Physicochemical and microbiological screening of open water reservoir collect from three different lakes in Bhopal

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ABSTRACT

Water is the second most essential requirement for life after air and plays a vital role in living organism and in various biological processes. It is used for multiple purposes such as drinking, agriculture, aquaculture, and industrial activities. The present study focusses on the analysis of water collected from 3 different lake in Bhopal Shahpura Lake, Arjun Nagar Lake, khatlapura lake. water sample were collected using the grab sampling method and analyzed for various physicochemical parameter such as Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), hardness of water (Calcium), PH, conductivity and solubility. Microbiological analysis was also performed, including preparation of culture media, colony count of bacteria and E. coli, and identification of the morphology of bacteria and fungi.

KEYWORD: BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), DO (Dissolve Oxygen), hardness of water (Calcium), Media Preparation, Colony Count, Bacterial Morphology Fungal Morphology.

INTRODUCTION

Water is the second most essential requirement for life after a living organism and in various biological processes. Water is used for many purposes such as drinking, agriculture, aquaculture, and industrial activities. Therefore, maintaining the quality and safety of water is extremely important for human health and the environment. water quality mainly depends on its physical, chemical and biological characteristics, this characteristic can be evaluated by measuring different parameters such as Ph, dissolved oxygen (DO), electrical conductivity biological oxygen demand (BOD) and chemical oxygen demand (COD). These parameters help in determining the purity of water and its suitability for drinking and another use. In recent years increasing industrialization and rapid population growth have placed great pressure on natural water resources. Many water sources become contaminated due to industrial waste, animal waste, and human human waste activities. Such contamination increases the amount of organic and inorganic substances in water and makes it a suitable medium for the growth of microorganisms.

Therefore, regular monitoring and analysis of water quality are very important the objectives of the present are to collect water samples from different lakes and analyze their physicochemical and microbiological parameters to assess water quality and ensure the availability of water.

REASON TO WORK ON WATER ANALYSIS

As a result, it is a formable factor and transmission of several diseases pollutant sewage water contains solid dissolved organic compound. The growth of multiplication of microorganism to linked to several diseases found in different types of water such as, fungi, protozoa, algae, bacteria and viruses. As per the 2016 WHO report globally at least 1.8 billons used fecal contamination of water drinking water resulting in 5 lakhs. Approximately 30,000 people died every day in developing country of the world because of unsanitary water supplied.

WHO AND UNICEF REPORT IN 2024- 2025

Indicate that while access to safely managed drinking water is rising, over 2 billion people still face contamination risks or lack safe service. Key threats include widespread bacterial pathogen (e.g., E. coli) ruler water system along with chemical contaminants like fluoride and nitrate.

SAMPLE COLLECTION

There are three different locations in Bhopal to collect water samples.

- Shahpura lake
- Arjun Nagar lake
- Khatlapura lake

Figure:1 Samples Collection

TABLE: SOME EXAMPLES OF MICROORGANISMS INCLUDE

S.NO	MICROBE TYPES	EXAMPLE NAME	DISEASES
1.	Bacteria	Salmonella Typhi/Vibrio Cholerae	Typhoid /Cholera
2.	Fungi	Micros Porum /Trichophyton	Fungal Infection/Ringworm
3.	Protozoa	Plasmodium/Amoeba	Malaria/Amoebiasis
4.	Virus	Influenza Virus /Rhinovirus	Flu/Common Cold
5.	Algae	Spirogyra Chlamydomonas /Cephalous	Red rust of (tea and coffee)

MATERIAL AND METHODS

1. DO (DISSOLVED OXYGEN)

Determination of the total dilution of water- There are two samples including in Dissolved oxygen Before incubator water sample and after incubator water sample.

Material- Water sample (A, B, C) sample A (Arjun Nagar), Sample B, (Shahpur a) Sample C (Khatlapura), Sodium Thiosulphate (0.25N), Mangus Sulphate, Alkaline Iodine Azide, Starch, Sulphuric Acid (conc.), BOD Bottle, 2ml pipette.

Method- Collect the water without bubbling in a water bottle, add 2ml of megnus sulphate and alkaline iodine iodide solution, Right the water bottle shakes inside down direction at least 6 times, Allow the precipitation so to add 2 ml of conc. H2SO4 and shake the bottle to dissolve the brown precipitation. Take a 50ml sample in a flask, 10ml with sodium thiosulphate solution the color change That 2 drop add the starch above the flask color of the content from till the blue color than titrant again with sodium thiosulphate then blue disappeared.

Before incubator sample reading in DO (dissolve oxygen)

To prepare alkaline iodide, (For 10ml), NAOH 5gm and potassium iodide 1.5gm sodium azide 0.1gm, Distilled water 10ml, After mix the NAOH and potassium iodide in 7-8 ml of distilled water. They are water bath in the solution at 80-90 centigrade in 20-30 min, after water bath boil and cool add the sodium azide and volume makeup of remaining distilled water, to prepare sodium thiosulphate 0.25N (normality), Molecular mass of sodium thiosulphate.

After incubator sample reading in DO (dissolve oxygen)

Collect the water without bubbling in water bottle, Added 2ml of megnus sulphate and alkaline iodine iodide solution, Right the water bottle shakes inside down direction at least 6 times, Allow the precipitation so add 2 ml of conc. H2SO4 and and shake the bottle dissolve the brown prescription, Take a 50ml of sample in a flask 10ml with sodium thiosulphate solution the color change That 2 drop add the thiourea above the flask color of content from till the blue color than titrant again with sodium thiosulphate then blue is disappeared.

2. BOD (BIOCHEMICAL OXYGEN DEMAND)

To calculate biochemical oxygen demand from dissolved oxygen, we used two types of samples, after incubation sample and before incubation sample after titration. When the reading of the sample was taken after and before we named it as D1 and D2. D1 is denoted by the initial value and D2 is denoted by the name of incubator water after incubation in 3 days. To calculate BOD, we subtract d1 from d2, which gives us our biological oxygen demand.

3. (COD) CHEMICAL OXYGEN DEMAND

Method

Take 3 sample in a conical flask and pour 50ml of water sample in each flask, Simultaneous run distilled water blank standard, Add 0.29gm/60ml of ($K_2cr_2O_7$) solution each of the flask, Keep the flask in water bath 100degree Celsius for 1 hours, Allow the sample to cool 10 minutes, add potassium iodide solution 7gm /70ml , Add 10ml of H_2SO_4 (2mol) 7.8ml /60ml in each flask ,Titrant the content of each flask with 0.1mol 5.688gm/360ml sodium thiosulphate until the appearance of pale-yellow color ,Add the 0.12gm/12ml of starch solution in each flask solution tense blue, The titrant in again with 0.1 m sodium thiosulphate until the blue color disappear.

COD in /mg = 8 × C × (B – A) / S

4. HARDNESS OF WATER (CALCIUM)

Calcium Hardness Water

Calcium hardness is determined by titrating in water sample in standard EDTA solution at PH -12 Magnesium precipitate only Calcium ions $(C)^{2+}$ remain in solution Calcium with EDTA to form a stable solution, Calcium EDTA complex. Phenolphthalein uses as an indicator.

Method

Take 25ml water into a conical flask, of 1 Normality of NaOH 4gm/10ml to rise PH, add small pinch of EBT (Eriochrome black tea), Titrate solution with 0.1 mole EDTA 1.752gm /60ml until color change purple or blue. Then read the Burette reading.

Formula

Calcium Hardness = V × M × 1000 / Volume of sample

5. **ISOLATION AND IDENTIFICATION OF BACTERIA-** Isolation and identification of bacteria, including *Escherichia coli*, were performed using selective and differential culture techniques. Samples were initially inoculated on Nutrient Agar Medium (NAM), a general-purpose medium that supports the growth of a wide range of non-fastidious microorganisms. Following incubation, distinct bacterial colonies were subcultured to obtain pure isolates.

For selective isolation and confirmation of *E. coli*, Eosin Methylene Blue (EMB) agar was employed. EMB agar acts both as a selective medium, inhibiting Gram-positive bacteria, and as a differential medium based on lactose fermentation. *E. coli* produces characteristic metallic green sheen colonies on EMB due to strong lactose fermentation, facilitating its identification.

This combined use of NAM and EMB agar ensures effective isolation, purification, and preliminary identification of bacterial species, particularly *E. coli*, in microbiological studies.

Methods

Measuring NaCl0.3gm/60ml, yeast extract0.18gm/60ml, peptone 0.3gm/60ml agar agar 0.33gm/60ml distilled water, in a flask of tryptone / peptone 0.3 gm, yeast extract 0.18 gm agar agar 0.3gm, Add the all of solution dissolved in 60ml of distilled water, Dissolved the media than autoclave at 15pci pressure 121degree Celsius for 15 minutes, The media done 3 into sterilize Petri plate under the sterilization condition.

Measuring peptone 0.6gm/ 60 ml, lactose 0.3gm, and dipotassium hydrogen phosphate 0.12gm and agar agar 0.9gm, add the all of solution dissolved in 60 ml of distilled water, dissolved the media than autoclave 50 pci pressure 121 degree Celsius for 15 mins, then the media done 3into sterilize petri plate under the sterilization condition.

6. **TO PREPARE LACTOSE BROTH**

Presumptive Coliform Test

The present team coliform test are used to detect coliform as a water sample in this test lactose fermentation tube are inoculate with different water volume and production of acid and gas from the fermentation of lactose and any the tube is a presumptive evidence and coliform in the water samples broth are used to test are selective for the isolation of coliform because of the addition to while and brilliant green a pH indicator purple is also added the lactose broth from the detecting acid the color are indicator change to yellow with production of acid from lactose.

Method

Collect the water sample lake and pond, Then label the five double and five single strength broth tube, Mix the water and shaking as hepatic inoculate 10 tube each 10 ml of water sample, Using at 1 ml of pipette ascetically with 1 ml of water sample, Using a 0.1 mole pipette aseptically inoculate with pipe tub with 0.1 of water sample, Incubate tube at 35-degree centigrade anaerobic condition at 48 hours .

Result and Observation

1. **DO Result**

Table:1 Before incubator sample reading in DO (dissolve oxygen)

S.NO	SAMPLE	Reading 1	Reading 2	Reading 3	Total /3	Mean(average)	Result
1.	Khatlapura	0.8	0.3	0.5	1.6/3	0.53	42.4
2.	Shahpura	0.6	0.3	1.1	2.0/3	0.6	48
3.	Arjun Nagar	0.9	0.3	0.9	2.1/3	0.7	56

Figure: 1Before incubator sample reading in DO

Table:2 After incubator sample reading in DO (dissolve oxygen)

S.NO	SAMPLE	Reading 1	Reading 2	Reading 3	Total /3	Mean(average)	Result
1.	Khatlapura	1.0	0.6	0.5	2.1/3	0.7	56
2.	Shahpura	0.5	0.4	0.3	1.2/3	0.4	32
3.	Arjun Nagar	0.2	0.4	1.1	1.7/3	0.56	44.8

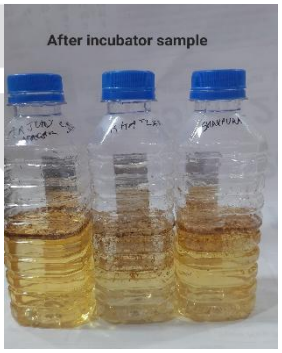

Figure: After incubator sample reading in DO

2. **BOD (Biochemical oxygen demand) Result**
   Table:3 Biochemical Oxygen Demand (BOD) Analysis of Water Samples from Different Locations

s.no	Sample	D1	D2	Result
1	Shahpura	48	32	16
2	Khatlapura	42.4	56	13.6
3	Arjun nagar	56	44.8	11.2

D/O= Initial value – after incubation in 3-day incubated water

3. (COD) CHEMICAL OXYGEN DEMAND

Table:4 chemical Oxygen Demand (COD) Analysis of Water Samples from Different Locations

S.NO	SAMPLE	Reading 1	Reading 2	Reading 3	Total /3	Mean(average)	Result
1.	Khatlapura	11	11.2	13.9	36.1/3	12.0	0.035mg/l
2.	Shahpura	10.5	11.7	9.5	31.7/3	10.5	0.011mg/l
3.	Arjun Nagar	15.5	14.6	17.5	47.6/3	15.8	0.098mg/l
4	Blank water	10	9.3	10.3	29.6/3	9.8	-

4. HARDNESS OF WATER (CALCIUM)

Table: 5 Determination of Calcium Hardness in Water Samples from Different Locations

sample	Khatlapura	Shahpura	Arjun Nagar	Distilled Water
Reading	5.0	9.0	7.0	1.9
Result	20	36	28	

Figure: 3 Determination of Calcium Hardness in Water Samples from Different Locations

5. Isolation and identification of bacteria

Table: 6.a Morphological character of NAM (Nutrient agar media)

S. no	Colony count	Sample A (Arjun Nagar)	Sample B (khatlpura)	Sample C (Shahpura)
1.	Types of colonies	3	2	3
2.	Margin	Entire, undulate, filament	Entire, filament undulate, round	Undulate, filament, lobate
3.	Shape	Circular, Irregular	Irregular	Irregular, filament rhizoid
4.	Color	White	White	White
5.	Surface	Rough	Rough /smooth	Rough /smooth
6.	Consistency	Dry	Dry	Dry
7.	Density	Translucent	Translucent	Translucent
8.	Elevation	Flate	Flate	am boned form

Figure: 4 Morphological character of NAM (Nutrient agar media)

Table: 6.b Morphological character of EMB (Eosin methylene blue)

Morphological character of EMB (Eosin methylene blue)

S. no	Colony count	Sample A (Arjun Nagar)	Sample B (khatlpura)	Sample C (Shahpura)
1.	Types of colonies	1	1	Blank
2.	Margin	Undulated	Entire	Blank
3.	Shape	Irregular	Regular	No growth
4.	Color	Brown	Brown	Brown
5.	Surface	Smooth	Smooth	Smooth
6.	Consistency	Dry	Dry	Dry
7.	Density	A peck	A peck	A peck
8.	Elevation	Umlauted	Flate	No growth

Figure:5 Morphological character of EMB (Eosin methylene blue)

6. TO PREPARE LACTOSE BROTH

Examine or lactose fermentation to produce acid gas of 24 and 48 hours for incubator.

Production of acid and gas after 24 hours incubation positive presumptive coliform bacteria, it is gas developed in tube after 48 hours of incubation, The presumptive test is doubtful, Note the record for two showing the positive presumptive test the tube showing present presumptive test the tube is showing all retained are used for coliform test.

Figure: 6 To Prepare Lactose Broth

Results and Discussion

The physicochemical and microbiological analysis of water samples from Khatlapura, Shahpura, and Arjun Nagar revealed variations in water quality parameters.

DO values are measured before and after incubation to evaluate oxygen consumption by microorganisms. The initial DO (before incubation) indicates the oxygen content present in the sample at the time of collection, whereas the final DO (after incubation) reflects the oxygen remaining after biological activity.

As shown in Table 1 (Before Incubation) and Table 2 (After Incubation), there is a noticeable decrease in DO levels after incubation for all samples:

Khatlapura: 42.4 → 56

Shahpura: 48 → 32

Arjun Nagar: 56 → 44.8

This variation in DO indicates microbial utilization of oxygen during incubation. The difference between initial and final DO values is directly related to the Biochemical Oxygen Demand (BOD) of the samples, which reflects the level of organic pollution in water.

Higher oxygen consumption suggests higher microbial activity and greater organic matter presence, indicating poorer water quality. Therefore, the comparison of DO values in Table 1 and Table 2 provides the basis for calculating BOD and assessing the pollution status of the studied water samples.

The **Dissolved Oxygen (DO)** values before and after incubation (Table 1 and Table 2) indicate oxygen consumption due to microbial activity. The decrease in DO after incubation confirms the presence of biodegradable organic matter in the samples.

The **Biochemical Oxygen Demand (BOD)** values (Table 3) were calculated using the difference between initial and final DO values. Among the samples, Shahpura showed the highest BOD value (16 mg/L), followed by Khatlapura (13.6 mg/L) and Arjun Nagar (11.2 mg/L). Higher BOD values indicate greater organic pollution and microbial activity, suggesting that Shahpura water is comparatively more contaminated.

The **Chemical Oxygen Demand (COD)** results (Table 4) further support the presence of oxidizable pollutants. Arjun Nagar exhibited the highest COD value (0.098 mg/L), followed by Khatlapura (0.035 mg/L) and Shahpura (0.011 mg/L). COD measures both biodegradable and non-biodegradable organic matter, indicating overall pollution load in water.

The **calcium hardness** analysis (Table 5) showed that Shahpura water has the highest hardness (36 mg/L), followed by Arjun Nagar (28 mg/L) and Khatlapura (20 mg/L), while distilled water showed negligible hardness. This suggests a higher concentration of calcium ions in Shahpura water, contributing to hardness.

Microbiological analysis using Nutrient Agar Medium (NAM) and Eosin Methylene Blue (EMB) agar (Table 6a and 6b) revealed the presence of diverse bacterial colonies. NAM supported the growth of

multiple colony types with varying morphology, indicating mixed microbial populations. EMB agar, a selective and differential medium, showed limited growth with characteristic colony appearance, suggesting the possible presence of *Escherichia coli* in some samples, while no growth was observed in Shahpura, indicating absence or low concentration of coliform bacteria.

Overall, the study indicates that all water samples contain varying levels of organic and microbial contamination. Based on BOD and COD values, Shahpura and Arjun Nagar samples show higher pollution levels, while microbiological analysis confirms the presence of bacterial contamination, emphasizing the need for proper water treatment before consumption.

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