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#### **Review Article**



# Microbial analysis of drinking water samples from different sources in Delhi

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# Abstract

People and ecosystems are threatened by health risks associated with poor water quality. Serious health disorders like diarrhea, dysentery, cholera, typhoid, etc. are caused by microbial contamination which is one of the major reasons for water pollution and poor drinking water quality. Physical, chemical, and microbial factors such as concentration of oxygen, amount of salt (salinity), or the amount of material suspended in water (turbidity), etc. are a few of the major qualitative parameters for safe drinking water. The health of water resources is getting worse all around the world and especially developing nations like India are the most adversely affected by it. India lacks both proper funding and means to deal with major issues like water pollution and the curses associated with it. There is an immediate need to work towards it because a country like ours with such a huge population is not ready to face water scarcity. It is of utmost importance to take necessary steps towards it both at the government and public level. This microbial study was performed in drinking water samples from different sources like Yamuna River water, municipality water, underground water, RO water, and packaged drinking water to analyze them for the presence of harmful microorganisms like *E. coli, coliforms, fecal coliforms, yeast, mould, pseudomonas, Salmonella,* and *Shigella.* The results indicated that Sample-1 Yamuna water was the most contaminated sample, followed by Sample-2 Groundwater. Sample-4 RO water was only contaminated by *yeast*, possibly due to a contaminated underground source. Sample-2 Municipality water was found to be completely contamination-free and fit for consumption due to chlorine treatment. No form of microbial contamination was found in Sample- 5 Packaged drinking water because of proper treatment under the BIS standards guidelines.

Keywords Water quality, microbial load, coliforms, Escherichia coli, fecal streptococci, salmonella, shigella.

## Introduction

Water scarcity is an issue that is affecting people all around the world. There is a significant increase in the global water demand of 1% per year. Factors like increasing population, economic development and change in consumption pattern are further aiding to the situation and are acting as catalysts in pushing the world towards water scarcity. The scenario doesn't seem to change for at least two more decades and would result in majority of the world facing adverse water scarcity. (WWAP 2018).

The situation becomes worse when water scarcity is coupled with high levels of chemical and biological contamination. Around 80% of the total wastewater produced in the world is directly dumped into the environment without treatment, polluting rivers and oceans and affecting millions due to the health hazards associated with it. Agricultural runoffs and unregulated industrial disposal also contribute to the increasing pollution levels. (MOEF 2016)) Untreated sewage and solid waste particles are the main cause of pollution in the rivers and total surface water in India (CPCB 2009).

River water is the major source of surface water for fulfilling the fresh water demands of the country. 351 river stretches were founded to be heavily contaminated by chemical and biological means in 323 rivers located in 29 states and 2 union territories in India. (CPCB 2008). River Yamuna along with River Ganga are considered to be the most polluted rivers in the country. Untreated Sewage disposal, Industrial waste disposal and pesticide runoffs from Delhi and other cities situated along the river are held responsible for the degrading water quality in the rivers. The river water is also highly contaminated by both coliforms and fecal coliforms due to discharge of untreated waste. Consumption of water loaded with coliforms lead to gastro- intestinal disorders like diarrhea, dysentery, viral and typhoid fevers (Anand et al., 2006) Apart from surface water, Groundwater is also a major source for fulfilling the water needs of the country. Out of the total groundwater available, around 89% is used for agriculture, 9%

by households and the remaining 2% by the industries. (CGWB 2015) Agriculture, Industrialization and urbanization are majorly responsible for the depleting underground reservoirs and degrading ground water quality. (British Geological Survey 2008) Groundwater is highly contaminated by heavy metals like fluoride, nitrate, arsenic etc. and high salinity. (Ngah et al. 2012) *Coliforms* and *faecal coliforms* also contribute to the microbial contribution of groundwater. (Kumar et al. 2014).

The major sources of water in Delhi is groundwater along with the Ganga Canal, the western Yamuna Canal, the Bhakra Canal and river Yamuna. Water and waste water management in Delhi is carried out by the Delhi Jal Board (DJB). (Economic survey of Delhi 2002) The water resources in the capital are highly contaminated by heavy metals and pathogenic microorganisms. (Kumar et al. 2016) Strategies should be made and implemented along with regular monitoring to improve the water related situation in the country and to build an effective water resource management.

#### **Material and Methods**

The testing was done following the Indian Standards (IS). Microbial testing of samples was done in 4 stages- Sampling, Testing, Characteristic colony Observation and counting, Biochemical confirmation.

# Sampling

Sampling was done following the IS 1622. 1.5-2 L of each sample were collected from 5 sources- River water- Yamuna water, Municipality water, Underground Water, RO water and packaged drinking water Sample 1. River water, 2. Municipality water from were collected at 3 PM on 1<sup>st</sup> March 2020 from Wazirabad; sample 5. Packaged drinking water was collected at 10 PM on 1<sup>st</sup> March from Rohini and sample 3. Underground water and 4. RO water were collected at 8 PM on 2<sup>nd</sup> March from Shastri Nagar as environmental samples should be tested within 24 hours of collection to freeze the microbial load.

# Testing

Testing of TPC was done using pouring technique and for other parameters membrane filtration was technique was used. Testing was started within 24 hours of sample collection. The samples were brought in the lab and stored at 0-4 degrees Celsius in a refrigerator. The samples were segregated as low risk samples and high-risk samples. Sampling for all Parameters- Qualitative and Quantitative was done simultaneously for all samples. Media controls and dilutions were also prepared. 100 ml of each water sample was filtered through filter assemblies in the Water laminar cabinet. Dilutions were made from 2-6 for samples with high microbial load as only 80 characteristic colonies can be counted on a filter. After filtration the filters were placed on the marked pre-prepared media plates using a sterile forceps.

# **Enumeration of Total Plate Count**

Enumeration of *TPC* was done following IS-1504. Five Dilutions for River Water, 3 for Municipality and Underground Water, 1 for RO Water and NO dilution for Packaged Drinking Water were made. After filtration, the filter was placed on Plate Count Agar (PCA). Incubation was done at 30 Degrees Celsius for 3 days.

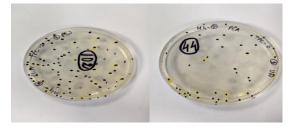


Fig. 1 Characteristic colonies of TPC

#### Enumeration of E. coli and Coliforms

Enumeration of *E. coli* and *Coliform* was done following IS 15185. Six Dilutions for River Water, No dilution for Municipality, 2 Dilutions for Underground Water and No dilutions for RO Water and Packaged drinking water were made. After filtration, the filter was placed on Chromogenic Coliform Agar (CCA). Incubation was done at 37 degrees Celsius for 1 day.



Fig. 2 Characteristic colonies of E. coli and Coliforms

#### Enumeration of Yeast and Mould

Enumeration of *Yeast* and *Mould* was done following IS 5403. Three Dilutions for River water, 1 dilutions for Municipality water, Underground Water and RO Water and No dilutions for packaged drinking water were made. After filtration, the filter was placed on Chromophenicol Yeast Extract Dextrose Agar (CYGA). Incubation was done at 25 degrees Celsius for 3 days for *yeast* and 5 days for *mould*.



Fig. 3 Characteristic colonies of Yeast and Mould

#### **Enumeration of Faecal Streptococci**

Enumeration of *Faecal Streptococci* was done following IS 15186. Two Dilutions were made for River water, 1 for Municipality, Underground and RO Water and No dilutions were made for Packaged Drinking Water. After filtration, the filters were placed on Slantez and Bartley medium (SBM). Incubation was done at 37 degrees Celsius for 2 days.



Fig. 4 Characteristic Colonies of Faecal Streptococci

# Enumeration of *Pseudomonas*

Enumeration of *Pseudomonas* was done following IS 13428. No dilutions were made (Qualitative parameter). Filters were placed in Asparagine Proline Broth for primary enrichment. Incubation was done at 37 degrees Celsius for 1 day. For secondary enrichment, a loop was dipped in the Aspargine Proline Broth bottle and streaked on Cooked Meat medium plate. Incubation was done at 42 degrees Celsius for 1 day.

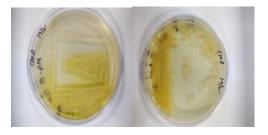


Fig. 5 Characteristic colonies of Pseudomonas

#### Enumeration of Salmonella

Enumeration of *Salmonella* was done following IS 15185. No dilutions were made. Filters were placed in Buffered Peptone Water for Primary Enrichment. Incubation was done at 37 degrees Celsius for 18 hours. For secondary enrichment, 0.1 ml sample was taken from buffered peptone water ottle and added to 10 ml RVSM tube and 1ml sample was taken and added to MKTTB tube. Incubation was done at 42 degrees Celsius for RVSM and at 37 degrees Celsius for MKTTB for 24 hours. A loop was dipped in the RVSM tube and streaked on both XLD and BGA plates. A loop was dipped in the MkTTB tube and streaked on both XLD and BGA plates. Incubation was done at 37 degrees Celsius for 1 day.

# Enumeration of Shigella

Enumeration of *Shigella* was done following IS 5887 part-7. No dilutions were made. Filters were placed in Nutrient Broth. Incubation was done at 37 degrees Celsius for 1 day. For

secondary enrichment, 1ml sample was taken from the nutrient broth bottle and added to 10ml SFB tube and 1ml sample was added to MKTTB tube. Incubation was done at 37 degrees Celsius for 1 day. A loop was dipped in SFB and streaked on DCA plate, and a loop was dipped MKTTB tube and streaked on DCA plate. Incubation was done at 27 degrees Celsius for 1 day.

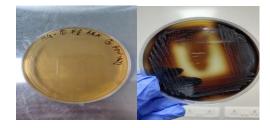


Fig. 8 *Faecal Streptococci* confirmation on Bile Aesculin Azide plates

## **Biochemical Tests**

Characteristic colonies were streaked on Nutrient Agar to obtain isolated colonies and incubate at 37 degrees Celsius for 1 day. For *E. coli*, Oxidase test was performed. Oxidase reagent (tetra methyl-p-phenylenediamine di hydrochloride) was rehydrated using distilled water and put on folded tissue paper. Loopful of colonies were applied to the tissue paper and observed for 10 seconds for colouration. No colouration was observed. Indole Test was performed. A loopful of colonies were taken and mixed in tryptone water and kovac's Reagent was added 18-24 hours later. Red ring formation was observed. Hence, *E. coli* (oxidase nedative and Indole positive) was confirmed.

For *Coliforms*, Oxidase test was performed. No colouration was observed. BGLB tubes with inverted Durham's tubes test was performed. Loopful were taken and mixed with the solution in the tubes and observed. Gas formation was observed. Hence, *Coliforms* (oxidase negative and BGLB tubes test positive) was confirmed.

For *Faecal coliforms*, **c**haracteristic colonies were streaked on Bile Aesculin Azide Agar. Incubation was done at 42 degrees Celsius for 1 day. Black colonies with transparent zone were observed. Hence, *faecal coliforms* were confirmed.

For *Pseudomonas*, oxidase test was performed. Blue colouration was observed. Hence, pseudomonas was confirmed.

# Results

Cfu for TPC was calculated using this formula- CFU/ml =  $(Sum of all the colonies counted)/(1^{st} dilution+0.1*2^{nd} dilution)*dilution factor.$ *TPC*count for Sample 1 was 11000cfu/ml. for sample 3 was 4400cfu/ml. For sample 2, 4 and 5 was <1cfu/ml.

For other parameters, membrane filtration technique was used and the cfu was calculated using this formula- CFU/100 mL = (No. of colonies counted  $\div$  sample volume filtered in mL) x 100. E. coli count for sample 1 was2600cfu/100ml. For sample 3 was 2500cfu/100ml. For sample 2, 3 and 5 was <1cfu/100ml. Coliform count of sample 1 was 78000cfu/100ml. For sample 3 was 320cfu/100ml. For sample 2, 4 and 5 was <1cfu/100ml. Yeast count for sample 1 was 800cfu/100ml. For sample 3 was 24cfu/100ml. For sample 2, 4 and 5 was <1cfu/100ml. Mould count of sample 1 was 260 cfu/100ml. For sample 2, 3, 4 and 5 was <1cfu/100ml. Faecal streptococci count of sample 1 was 2000cfu/100ml. For sample 3 was 300 cfu/100ml. Pseudomonas was present in sample 1, 3 and 4. Salmonella was absent in all the samples. Shigella was also absent in all the samples.

#### **Discussion and Conclusion**

The present study was conducted to analyze the microbial quality of the five major drinking water sources in Delhi. It has been well established that the river water and underground water are the two major sources for all kinds of water supplies and are still heavily contaminated by both microbial and chemical means. Hundreds of crores of rupees have been spent to improve the water quality of Indian rivers but the issue needs to be addressed from the source only i.e. the discharge of Industrial and Municipal waste into water bodies. The underground water is mainly polluted by the breakage and leakage in sewage lines. Underground water is the major source of water for the Municipality supplies, RO purifiers and packaged drinking water supplies. Regular reports addressing the poor municipal supplies and RO water contamination is a sufficient proof that these sophisticated technologies and Chlorine treatment are not enough to deal with the high contamination levels. Packaged drinking water is obviously considered the safest and most contamination free option but it is not practical nor economical to use it regularly for all purposes. Also, only the big companies with proper certifications ensure the quality as small companies supplying and packing water are alleged of malpractices at regular intervals.

It is known that majority of people in Delhi don't consume Yamuna water directly but people living near the river or the people that take religious baths in the river or consume little quantities of it as religious practices are susceptible to the health hazards associated with it. Underground water is considered to be a good and safe source of drinking water because water that seeps through the various layers of ground gets purified on its way down to the reservoirs. But due to the sewage pipelines spread throughout the city, a single leakage can contaminate the water unless water is pulled from really deep into the ground. But Delhi being the city of 1.9 crore people puts a lot of pressure on the underground water reservoirs. Industries, Municipality corporations and Households together depend largely on the underground water for fulfilling the water demands. The burden gets even bigger because the replenishment rates are much lower than the rate at which water is extracted out. Despite of reports about the

bad drinking water quality provided by the Delhi Municipality Corporation or the Jal Board, this study strengthens the fact that the Municipality water is relatively contamination free, economical and readily available. Majority of the people in Delhi can't afford an RO purifier or packaged drinking water to fulfil their potable water needs and should resort to Municipality supplies because its contamination free and absolutely fit for consumption. Mostly the primary source for RO purifiers is groundwater. According to the study, they are not completely effective in making the water contamination free and to ensure proper purification, the filter membrane should be changed regularly. But in areas with extremely high contamination levels, they might not prove to be very effective. Also, the water wasted in the purification process is a major concern and therefore, use of RO purifiers in areas with TDS lower than 500 was also prohibited. Use of RO filters with very low TDS also eliminates necessary minerals like calcium. Water is a precious resource and wasting it is completely unacceptable and hence RO filters should only be used in areas with high TDS and contamination levels. Packaged drinking water specially branded one which has a good reputation in the market proved to be completely contamination free and absolutely fit for consumption. Due to the popularity and trust of public in the brand, lot of fake products under the same or similar name are sold in the market. These products don't have any proper certification neither fulfil the required parameters and should not be consumed to avoid health associated risk and wastage of money.

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