

# Physicochemical Characterization and Microbial Analysis of Commercial Drinking Water, Chennai, Tamil Nadu

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## Abstract

This research focused on the physical qualities, chemical composition and microbiological contamination of various brands of bottled water sold around Chennai city, Tamil Nadu, India. Aims and Objectives of the present investigation is to determine the physicochemical parameters such as color, odor, pH, EC, BOD, COD, chloride and fluoride of the different water samples of commercial bottled drinking water and microbial analysis were also studied for the water samples. The results of the study revealed that the parameters of the sample were within the permissible limit as prescribed by WHO guidelines. Bacteria such as *Bacillus subtilis*, *Klebsiella Pneumoniae* and fungal species such as *Mucor* sp and *Aspergillus* sp were identified in the water samples thereby indicating that the water is contaminated, which may cause infection and water borne diseases.

**Key words:** Commercial bottled drinking water, Physicochemical parameters, Microbial analysis, Contamination, Infection

Water is a necessity for all living things in this world. Water has a variety of purposes for humans, animals and other living organisms. Drinking water that is clean, safe and hygienic is essential for good health. Drinking water should be potable, which means it may be consumed in any volume without causing health problems or endangering human life. The quality of water has deteriorated as a result of sloppy waste disposal, sewage, poor water management and environmental irresponsibility [1]. Water quality can differ from one source to the other depending on a variety of factors such as water sources, water purification methods and storage tanks [2]. There are no strict quality control requirements in place for bottled water used for human consumption. Usage of commercial drinking water by the public has become more frequent. Many people drink on a daily basis. They would not know that consuming commercial drinking water causes water-borne diseases. Packaged water bottles have a number of microbes that cannot be seen with the naked eye. These microbes cause serious health issues for those who consume it. Based upon the above views, the present investigation was carried out to determine Physicochemical parameters and microbial analysis of the commercial bottled drinking water.

10 different Commercial bottled drinking water samples (I, II, III, IV, V, VI, VII, VIII, IX and X) were selected for this project and collected in sterilized plastic bottles of 500 ml and 1000 ml capacity. Commercial bottled drinking water was procured from the shops in Chennai, Tamil Nadu, India. The samples were collected freshly as soon as the bottles were opened and brought to the laboratory for further analysis.

### *Physicochemical parameters of water sample*

Physicochemical parameters of water samples such as pH, EC, BOD, COD, Chloride and Fluoride were carried out by following the Standard Methods of [3].

### *Isolation of microorganisms from water samples*

#### *Stock culture*

1 ml of different water samples were taken and transferred into a conical flask containing 99 ml of sterile distilled water. Then it was shaken and serially diluted.

### *Nutrient agar medium preparation*

Nutrient agar medium was prepared by dissolving 1gm of nutrient agar in 100 ml of distilled water and sterilized. After sterilization, the medium was poured into sterile petri plates and were allowed to solidify for 30 minutes.

## MATERIALS AND METHODS

### *Collection of samples*

### *Pour plate method*

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1 ml of serially diluted different water samples were cultured in sterile petri plates which contain nutrient agar medium following pour plate method after which they were inverted and incubated for 24 to 48 hours at 37°C. After the incubation period, bacterial colonies developed on the medium in the plates were determined by following the procedure of [4-5].

#### Malt extract agar medium preparation

Malt extract agar medium was prepared by dissolving 1gm of malt extract agar in 100 ml of distilled water and sterilized. After sterilization, the medium was poured into sterile petri plates and were allowed to solidify for 30 minutes.

#### Pour plate method

1 ml of serially diluted different water samples were cultured in sterile petri plates which contain Malt Extract Agar medium (MEA) using pour plate method and incubated at room temperature for 4-5 days. Fungal species developed on the medium were observed periodically using lactophenol cotton blue by following the Manual of [5] and identified.

#### Identification and characterization of microorganisms

##### Identification and characterization of bacteria

Microorganisms that were identified as *Bacillus* sp, *Klebsiella* sp. Dominant microorganisms were characterized and confirmed by biochemical tests such as Indole test, Methyl red test, Voges-Proskauer test, Citrate Utilization test, Triple Sugar Iron test, Urease test, Catalase test, Coagulase test,

Nitrate reduction, Gelatin hydrolysis. The dominant bacteria were selected based on colony counting and tested for gram staining and biochemical tests and they were identified as *Bacillus subtilis* which is gram positive and showed positive results for Gelatin hydrolysis, Citrate test, Voges Proskauer test and Nitrate reduction test. The other dominant bacteria identified as *Klebsiella pneumoniae* is gram negative and showed positive results for Catalase test, Urease test and Triple Sugar Iron test.

## RESULTS AND DISCUSSION

#### Physicochemical parameters of different commercial bottled drinking water

The data obtained from the above experiments were expressed as Mean, Standard deviation and One-way ANOVA. The results for the physicochemical characteristics of different water samples (Table 1) revealed that the color of water is colorless, the taste of water is little bit sweet and odorless, the pH of water is around 7.120±0.084 to 7.140±0.167, EC value ranged from 48.8 ±22.26 µmhos/cm to 79±51.52 µmhos/cm. The BOD value of water ranged from 1.48±0.228 mg/l to 1.8±0.212 mg/l. The COD value of water ranged from 2.76±0.207 mg/l to 2.9±0.255 mg/l. The chloride in water ranged between 5.88±1.171 mg/l to 5.94±2.552 mg/l. The fluoride in water has a minimum value of 0.058±0.0164 mg/l and maximum value of 0.058±0.0164 mg/l. The data obtained for One-way ANOVA revealed that BOD is Statistically significant which is p<0.05.

Table 1 Physicochemical parameters of different commercial bottled drinking water

Parameters	Mean ± Standard Deviation	F Value	p Value
pH	7.120 ± 0.084	0.0572	0.8171
	7.140 ± 0.167		
EC µmhos/cm	48.8 ± 22.26	1.4476	0.2633
	79 ± 51.52		
BOD mg/l	1.48 ± 0.228	5.26	0.0506
	1.8 ± 0.212		
COD mg/l	2.76 ± 0.207	0.9071	0.3688
	2.9 ± 0.255		
Chloride mg/l	5.88 ± 1.171	0.0023	0.9631
	5.94 ± 2.552		
Fluoride mg/l	0.058 ± 0.0164	0	1
	0.058 ± 0.0164		

\*p<0.01 and \*p<0.05

Table 2 Identification of bacteria and fungi species in commercial bottled drinking water

Bacteria	Fungi
<i>Pseudomonas</i> sp	<i>Mucor</i> sp
<i>Bacillus</i> sp	<i>Penicillium</i> sp
<i>Klebsiella</i> sp	<i>Aspergillus</i> sp
<i>Salmonella</i> sp	<i>Rhizopus</i> sp

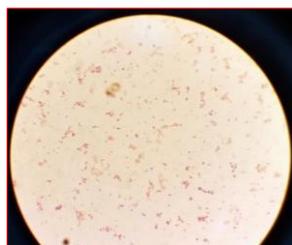


Fig 1a Gram staining for *Bacillus subtilis*



Fig 1b Gram staining for *klebsiella pneumoniae*

Table 3 Biochemical tests for dominant bacterial species

Biochemical Tests	<i>Bacillus subtilis</i>	<i>Klebsiella pneumoniae</i>
Gram Staining	+	-
Shape	Rod	Rod
Indole Test	-	-
Methyl Red Test	-	-
Citrate Test	+	+
Triple Sugar Iron Test	-	+
Voges Proskauer Test	+	+
Urease Test	-	+
Nitrate Reduction Test	+	+
Gelatin Hydrolysis Test	+	-
Catalase Test	+	+

+ Positive

- Negative

The results of microbial analysis of different water samples (Fig 1a-b), (Table 2a-b) revealed the presence of *Bacillus subtilis* (Fig 2a) and *klebsiella Pneumoniae* (Fig 2b). Whereas fungi present in commercial drinking water were identified as *Mucor* sp and *Aspergillus* sp.

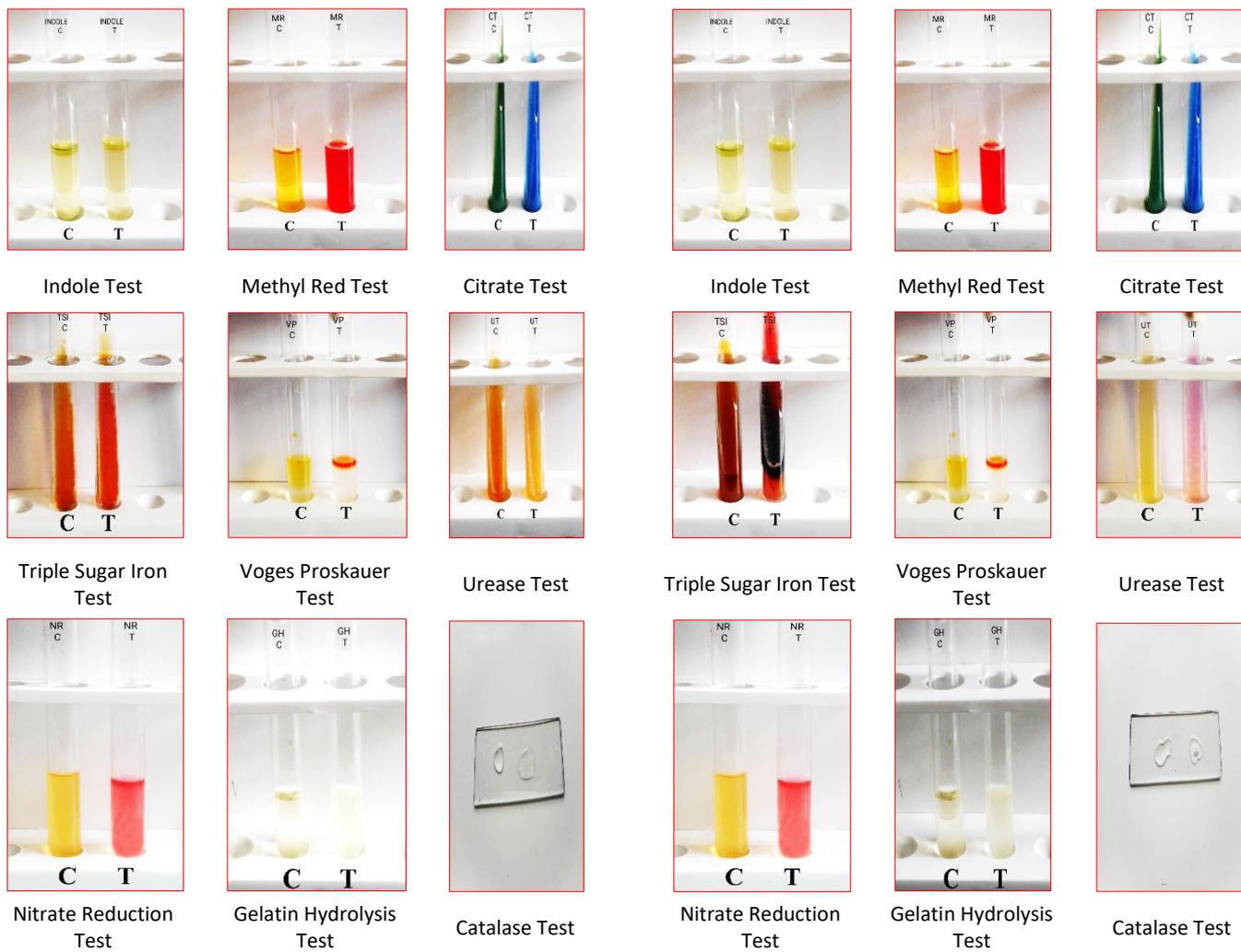


Fig 2a Biochemical Tests for *Bacillus subtilis*

Fig 2b Biochemical Tests for *Klebsiella pneumoniae*

Commercially available bottled drinking water contaminated with harmful bacteria and fungi can seriously harm people and result in cholera, typhoid fever, and other gastrointestinal illnesses. pH of water is the most important factor in determining its corrosiveness. The lower the pH value, the more corrosive the water [6]. pH is also linked to electrical conductance and total alkalinity [7]. In this study, the pH is ranged between  $7.120 \pm 0.084$  to  $7.140 \pm 0.167$  which is within the permissible limit as per [8].

Electrical conductivity is a measure of an aqueous solution's ability to carry an electric current and is affected by the presence of ions, their total concentration, mobility and temperature [9]. In this study, the EC ranged between  $48.8 \pm 22.26$   $\mu\text{mhos/cm}$  to  $79 \pm 51.52$   $\mu\text{mhos/cm}$  is within the permissible limit of [10] Hence it is suitable for drinking and the results of this study is supported with the work of [7].

The amount of oxygen required by bacteria to stabilize decomposable organic matter is commonly defined as Biochemical Oxygen Demand. BOD indicates the extent of pollution of the water samples [11]. Water with BOD levels  $<4$  mg/l are deemed as clean, while those  $>10$  mg/l are considered polluted and unsafe [12]. COD is a chemical oxidant that is used to measure the oxygen equivalent of organic matter in a sample. COD values should be less than 10 mg/l after water treatment. The BOD values of bottled drinking water in this study were between  $1.48 \pm 0.228$  mg/l to  $1.8 \pm 0.212$  mg/l which is less than 4 mg/L [8] and the COD value between  $2.76 \pm 0.207$  mg/l to  $2.9 \pm 0.255$  mg/l, which is less than 10 mg/l considered to be potable drinking water [11].

Chloride can be found in varying concentrations in all natural waters. As the mineral content increases, likewise does

the chloride content [12]. High chloride content in water samples may be due to pollution from rich effluent of sewage and municipal waste, but chloride in excess imparts a salty taste to water and causes laxative effects in people who are not accustomed to high chloride levels [13]. In this study, the chloride content present in the bottled drinking water ranged between  $5.88 \pm 1.171$  mg/l to  $5.94 \pm 2.552$  mg/l which were within the permissible limit (250 mg/l) of [8]. The results of this study correlate with the work of [10].

Fluoride content in the bottled drinking water is between  $0.058 \pm 0.0164$  mg/l to  $0.058 \pm 0.0164$  mg/l which is within the permissible limit of [8] and thus the findings coincide with the work of [14]. Fluoride is required by humans as a trace element and higher concentrations of this element are toxic. A fluoride concentration of 0.6-1.0 mg/l in potable water prevents tooth decay and promotes bone development [15]. Thus, the physico-chemical parameters of the commercial drinking water are all within the permissible limit of [8].

Microbial contamination in the commercial drinking water samples were also carried out. The results of the study showed the presence of bacteria and fungi. There should be absence of bacteria and fungi in drinking water. The results of this study showed that the different water samples are contaminated with various species of bacteria and fungi. The bacterial coliforms present in commercial drinking water were identified as Gram-positive *Bacillus subtilis* and Gram negative *klebsiella pneumoniae* found to be present in all the 10 different water samples. The results of the study were supported by [16-17].

The major fungi genera found in Norwegian drinking water were *Penicillium* spp., *Absidia* spp., *Acremonium* spp.,

*Aspergillus* spp. and *Mucor* spp. Some of the species isolated from water samples are known to be strong allergenic skin irritants or to cause infections in immunocompromised people such as AIDs, cancer and organ transplant patients as well as people with asthma or other respiratory problems [18]. The result of this study revealed the presence of *Aspergillus* sp and *Mucor* sp were in the 10 different water samples which correlates with the work of [19-20]. Potable drinking water is a clear liquid that has no color, taste or odour. However, when infected with organisms such as fungi, these qualities are lost and the water becomes harmful to both human and animal populations [21-23].

## CONCLUSION

Thus, it can be concluded from the results of the above study that water is an essential source for living things.

Commercial bottled drinking water may cause harmful effects and water borne diseases. Hence it is contaminated with microbes as evidenced from results of the present study. So, this project is an awareness to the public who consume commercial bottled drinking water. People should know that bottled drinking water is not safe to drink because it contains microbes that cause health issues. So, we should stop drinking commercial bottled drinking water and take our own water bottles wherever we travel. Drinking boiled water is safe and avoid bacterial and fungal infection and water borne disease.

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