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Article

Microbiological and Physicochemical Evaluation of Packaged Drinking Water Marketed in Kadapa, Andhra Pradesh, India

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ABSTRACT

Safe drinking water which is reliable and reasonably priced is a human right and is necessary to maintain human health. Constant raise in the sale and indiscriminate use of packaged drinking water is of public health significance. The present study used analytical procedures and compared with Bureau of Indian Standards (BIS) drinking water specification to evaluate the physicochemical and microbiological characteristics of locally available 25 packaged water brands from different batches. Overall, 25 different brands (17 bottles and 8 sachets) were analyzed for presence of bacterial indicators of water quality. Total heterotrophic plate counts range from 0 to 140 CFU for water brands. Total three brands of water samples were deviating from the BIS standards. Total coli forms and *Escherichia coli* were noticed in only two brands of water samples. Attempts need to be strengthened in the examining of activities in this rapidly expanding industry with a view to raising standards.

Key words: Physicochemical quality, Coliforms, Sachet and bottled water, Heterotrophic plate count, Bureau of Indian Standards.

1. INTRODUCTION

Pure drinking water is the most vital essence in human evolution and also is a fundamental part of our life and health in particular. Increase in population and industrialization, the demand of the freshwater increases in the past few decades. Naturally, the demand for fresh water for human activities were attained from different sources like surface water, that is, rivers, springs, and un protected wells, piped water, bore-wells, or rainwater, and purified water in bottles [1]. As per the World Health Organization (WHO), Central Pollution Control Board, Bureau of Indian Standards (BIS), Indian Council of Medical Research, and Niti Aayog overall, 70% of the water in rivers were found to be contaminated and some of river water was too poor for human consumption [2] and out of 122 countries India ranked 120 in terms of water quality. A large volume of improperly treated domestic sewage, over use of fertilizers and pesticides from agricultural lands, untreated industrial effluents, and landfills are the key sources of contaminants in the polluted river water. Recently, the WHO estimated that 60% of deaths are due to diarrhea, out of which 35% are due to scarcity of pure drinking water, 31% due to poor sanitation, and 12% due to poor hygiene in middle-income countries [3].

According to the WHO and United Nations International Children's Emergency Fund, 2021 right to use safe drinking water that is reliable and reasonably priced, is a human right and is necessary to maintain human health [4]. Moreover, in modern era, contemporary lifestyle insists a higher quantity of freshwater needed to maintain living standards and to prevent the water-born diseases. On the other hand worldwide, there were still 2 billion people, who do not have access to safe drinking water in 2020. In addition to that, the target of achieving sustainable development goal 6 by 2030 is a dream because it needs a quadrupling of present progress rates in potable water services. As a response to this challenge, the current years have witnessed the appearance and incredible development of packaged drinking water (PDW) industry [5]. The sensible cost, improved taste, and free from pollutants have made that PDW is only the option of drinking water in majority of countries. According to the Trade Promotion Council of India (TPCI) in India, the PDW market cost was valued at $24 billion in 2019, and by the end of 2023, it was anticipated to reach $60 billion. The main reasons behind that the extreme growth of packaged drinking water industry is, due to the poor water quality provided by municipality and the public opinion is that the bottled water is essentially of good quality. However, many studies have revealed that the trust by public is need not always be true. According to the (National Research Development Corporation) study, more than 25% of brands of purified water is really just tap water in bottle, sometimes treated and sometimes not.

Today, PDW industry is one of India's fastest expanding industries and positioned among top ten countries in consumption of potable drinking water (The Indian bottled market 2012). Different countries have led the enforcement of water standards and recommended the maximum acceptable limits of various elements. Therefore, Indian Union Ministry of Health and Family Welfare released a notification on September 29, 2000, for all packaged water manufacturers and traders, based on International Statistical Institute certification from Bureau of Indian Standards (BIS) was made mandatory. The majority of reports in this area were carried out, on parameters like physicochemical or bacterial quality separately; very scanty reports found, on the collective quality assessments of both physical chemical and bacteriological quality of bottled water and sachet water. Hence, the present study was considered with the aim of assessing the physicochemical and microbial quality of

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sachet water and bottled water which were sold in Kadapa district with specified Indian standards.

2. EXPERIMENTAL

2.1. Sample Collection

The present study was carried out in Kadapa city of Andhra Pradesh state situated in south India. The samples were collected from various public and commercial places in Kadapa. Samples were numbered 1 to 25. The selected bottles and water sachets were verified for leakages, good capping, and its protective sealing in case of bottle before purchase. Twenty-five brands (17 bottles and 8 sachets) of water samples were analyzed. Duplicate batches of each of the brands were purchased randomly and transported into cool boxes to the laboratory for analysis.

2.2. Analytical Methods

The analytical methods were adapted from the BIS standard IS 14543: 2016 guidelines which are covered in the relevant Prevention of Food Adulteration Act of the Government [6].

2.2.1. Physical parameters

The collected water samples were analyzed for physical parameters such as temperature, pH, color, odor, taste, and total dissolved solids (TDS) as per the guidelines given in the BIS manual.

2.2.2. Microbial parameters

The bacteriological analysis was performing for both the qualitative and quantitative estimations of specific possible microbes present in the water samples. It consist tests for total viable count which includes both the bacterial and fungal populations, as well as for total coli form counts according to the BIS. Extreme care must be taken during the microbiological analysis of water samples so that the external contamination of the sample would be avoided.

2.2.2.1. Standard plate count

Dispense aseptically 0.1 mL and 1 mL of water samples in to sterile Petri plates and immediately 15–20 mL of sterile and cooled up to 40°C plate count Agar media was dispensed in to each Petri plate. The plates were mixed properly by rotating the plates in clock wise and anticlock wise direction and allowed the plates for solidification. Then, plates were incubated in inverted position at 37 ± 1°C for 48 h. After 48 h, the total number of bacterial colonies was counted by colony counter.

2.2.2.2. Yeast and Mold Count

To determine the yeast and mold counts present in the water samples, membrane filter technique was used [7]. Total 100 mL of samples was filtered with membrane filters by 0.45 μm pores (Millipore, Massachusetts, USA); then, the membranes were placed on Sabouraud dextrose agar (Himedia) medium which is supplemented with streptomycin at the concentration of 50 μg/ml; and then, plates were incubated at 28°C ± 1°C for 3 days in inverted position in BOD incubator. The total number of fungal colonies was counted after incubation by colony counter.

2.2.2.3. Enumeration of coli forms

Multiple tube fermentation method was employed to determine the fecal coli forms (FC) present in the sample [7] and statistical table (MPN index table) was used to interpret the results. The bacterial count present in the test samples was expressed as colony- forming unit (CFU) per 100 ML

MPN method was conducted in three stages, that is, presumptive coli form test, confirmed test and completed test. In presumptive coli form test, a total of 15 tubes (three sets each consists of five tubes) five of 10 mL of water sample was inoculated into each of 10 mL of lactose fermentation broth in double strength and 5 of 1 mL and 0.1 mL of water samples were inoculated in to each of fermentation broth with single strength medium and incubated at 37°C/48 h. Bromocresol purple and inverted Durham tubes were inoculated in to each of the tubes. The color change of medium in to yellow and collection of gas in Durham tubes was considered as positive for presumptive coliform test. If the water sample gives presumptively positive results, concurrent inoculation into brilliant green lactose bile broth (Difco, Maryland, USA) for total coli forms and EC broth (Difco, Maryland, USA) for fecal coli forms (FC) was inoculated. Cellular growth for EC broth incubated at 44.5°C for 24 h was considered as positive completed test. Similarly, positive brilliant green lactose bile broth with negative EC broth cultures indicated the presence of non-fecal coli forms.

3. RESULTS AND DISCUSSION

A total of 25 packaged drinking water samples which included both the bottles and sachets in the present study. In this study, mainly we concentrated on the microbiological quality of the collected water samples which include heterotrophic plate count (HPC), yeast, and mold count, and further, the samples were analyzed for the gram negative and fermentative group to evaluate the fecal contamination by MPN technique. Apart from the microbial quality of the samples, we also included the some of physicochemical parameters which include color, odor, taste turbidity, pH, and TDS.

3.1. HPC

Figure 1 shows that the aerobic bacterial count present in various brands of water samples. Total count from 0 to 140 cfu/mL. Water brands 1, 2, 5, 8, 11, 13, and 23 samples do not have any colonies in both the plates (sample volumes 0.1 and 1 mL). Samples 4, 9, 10, and 15 do not show any bacterial growth in 0.1 mL volume and found the some colonies in 1 mL volume, whereas 6, 7, 12, 14, 16, 17, 18, 19, 20, 21, and 22 samples less colonies in both the volumes. Almost all brands except 3, 24, and 25 samples contained HPC counts lower than the suggested by BIS (1×10^2 cfu/mL), while these sample elevated CFUs than the recommendation by the BIS (35, 29, and 30 colonies in 0.1 mL samples whereas 110, 120, and 140 in 1 mL sample).

3.2. Yeast and Molds Count

Twenty-five drinking water brands tested three brands (3, 24 and 25) of water samples contaminated with fungi [Figure 2]. Mycelium showed non-septate hyphae with broad, irregular walls and branches that form more or less at right angles was noted under the microscope. This indicates that brand 3 and 24 had *Mucor* spp. Brand 25 had colonies with powdery appearance. The hyphae were branched and septate, this indicates that these brands had *Aspergillus* spp. as contaminant.

3.3. Total Coli-Form Count

Presumptive test was carried out to the suspected water samples with high bacterial counts to heterotrophic bacterial counts. Bottled water brands 3 and 25 shown positive results (both gas and acid production) (Table 1). This indicates the possible contamination of coli form bacteria. Except the brands 3, and 25 remaining all brands showed negative results. In presumptive test, coliform bacteria produced both gas and acid. Hence, it was confirmed that except three brands remaining all the brands included in the present study were not have any coliform bacteria. Existence of coliform contamination was confirmed by conducting conformed and completed tests, and further, it was conformed the organism by biochemical characterization. The water samples produced gas and acid at 37°C within 24h or 48 h, is considered as the positive samples, which indicates the presence of normal coliform bacteria.

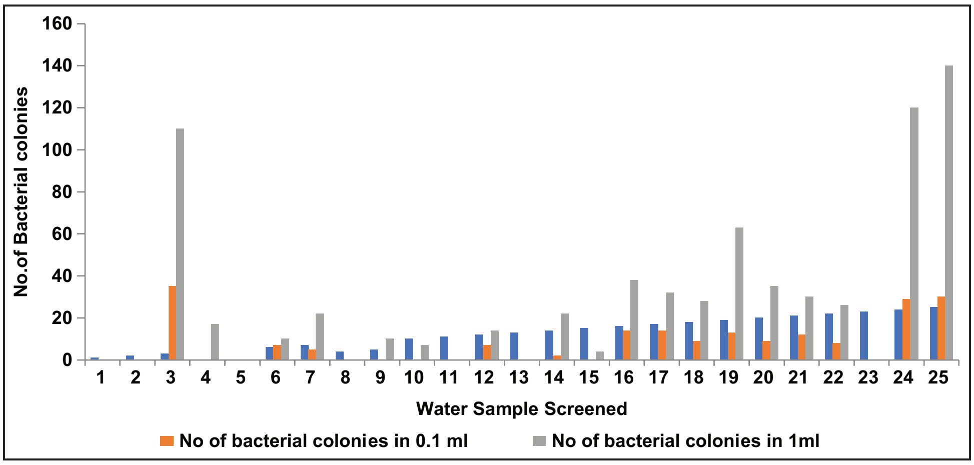

Figure 1: Heterotrophic plate count for the samples.

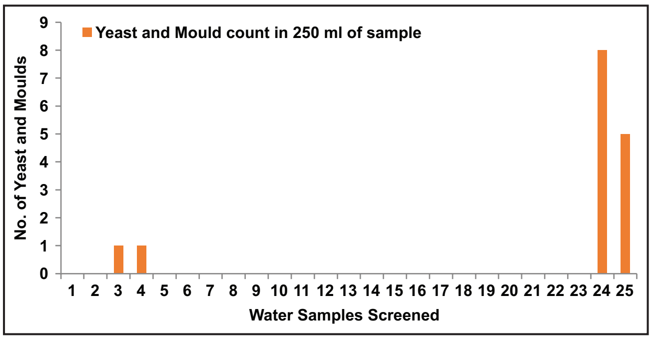

Figure 2: Yeast and mould count for the samples.

The revival of HPC beyond the considerable range in the present study was lower than other reports. In bottled water, samples contaminated with 92% and 59.4% were reported in Dar es Salaam, Tanzani and Lebanon by Kassenga [8] and Semerjian [9], respectively, and higher than the study in Egypt by Abd El-Salam (30.9%) [10]. The highest counts of HPC reported in the present study were similar to Majumder in Dhaka, Bangladesh [11]. The higher levels of HPC in the drinking water may designate the importance of treatment and safeguarding of bottled drinking water systems [12] and the presence of probable pathogens, particularly in immune compromised ones [13]. The presence of HPC in high numbers due to contaminated bottles used in bottling and malfunction of ozonation or UV apparatus or contamination of water by workers [14].

The contamination of mold (35%) in bottled drinking water in the present study was lower than the reports of Yamaguchi *et al.* [15] and also noticed yeasts in 36.6% of the samples which are much lesser than the present study. Although, in a study conducted [10], there was no bottled water contamination by any fungi [11]. The presence of mold and yeast shows a process safety and the intensity of quality control [16].

As per the WHO, diarrheal diseases account for an expected 4.1% of the total daily global burden and are liable for the deaths of 1.8 million people per year. It was expected that 88% of that burden is caused by unsafe water supply, sanitation, and hygiene [17]. In the present study, out of 25 water samples, 8% (2/25) had more than ten total coliform per 100 mL of water. Ideally, there should be no coliform per 100 ml of treated water based on the WHO instructions for drinking water and 92% of the water samples fell within this standard. Hence, 8% of the water samples (two of the 25 brands) failed to meet the BIS drinking water standard of zero coliform per 100 mL making them not suitable for human use. The presence of indicator organisms shows that water is contaminated by dangerous fecal matter, and hence, their absence indicates that the water is safe. Although, coliform may not for all time be directly related to the presence of fecal contamination in drinking water, the coliform test is still useful for monitoring the microbial quality of drinking water [18]. Malfunctioning of the treatment method employed could also result in the presence of coliform in the water samples. Suitable treatment methods should be used for production of quality and safe packaged drinking water [19].

The indicator bacterium *E. coli* is still used as target bacterium. The screening methods for the presence of indicator organisms were established with fermentation of lactose by production of acid and gas at 37°C. Although, waterborne disease causing organisms such as *Salmonella* and *Shigella* are not detected by the standard screening tests. The standard of water for drinking allows absolutely contamination free water (0 coliform/100 mL) [20]. However, only three brands, that is, 3, 24, and 25 of the investigated water samples (8%) were not appropriate for consumption. Other forms of bacteria, such as *cocci,* were also found in some of the brands. The occurrence of total coli forms in 8% of the samples of bottled drinking water in the present study indicates the presence of pathogenic enteric microbes, improper water treatment [21], and handling and purification procedure, washing and filling of the bottles due to poor hygienic, hand hygiene, illiteracy, and unhygienic practices of vendors [22]. Different studies employed by Ahmed *et al*. [23] and Gangil *et al.* [24], India reported maximum amount of total coli forms, thermostable coli forms, and *Escherichia coli* in bottled water. While in Vikarabad, Telangana, India, Rao *et al*. [25] and Singla *et al*. [26] in Delhi, India revealed that all the coliforms not detectable.

This was related to the results of another research done in Mangalore in 2002 which observed 66.7% of the sampled bottled water safe for consumption. This means that the condition of hygienic position of bottled water available was not shown any improvement with time. It may be the reason that, this issue was not given right of way as much as other public health issues concerning. In some other studies conducted in Indian scenario, the acceptability range of HPC counts of packaged drinking water is arrayed from 60% [24, 27], 83% [28], and even 100% [25,26,29].

In studies done in other parts of Asia, the acceptability of bottled mineral water samples array from 50% [30], 64.2% [31], and 97.1% [32] and a study done in Iran even reported 100% [33]. In studies done in Africa, it was 67.4% [22], 70% [34], 71.4% [35], 75% [36], 88.9% [37], 90% [38], 94% [39], and a study in Uganda [40] and Nigeria [41] reporting 100%.

Table 1: pH and Total dissolved solids of the study samples.

Name of the Sample	pH	TDS
1	7.8	082
2	7.6	210
3	7.6	225
4	7.4	216
5	7.0	205
6	5.9	047
7	6.8	245
8	6.4	247
9	7.0	228
10	7.4	024
11	7.8	226
12	7.2	243
13	7.4	243
14	7.4	063
15	6.9	232
16	7.2	218
17	7.6	224
18	7.4	232
19	7.6	228
20	7.2	218
21	7.4	226
22	6.8	232
23	7.4	205
24	7.9	095
25	6.9	243

Table 2: Physico-Chemical properties of the selected water samples

Name of the Sample	Color	Odor	Taste	Turbidity
1	Normal	Agreeable	Agreeable	Nil
2	Normal	Agreeable	Agreeable	Nil
3	Normal	Agreeable	Agreeable	Nil
4	Normal	Agreeable	Agreeable	Nil
5	Normal	Agreeable	Agreeable	Nil
6	Normal	Agreeable	Agreeable	Nil
7	Normal	Agreeable	Agreeable	Nil
8	Normal	Agreeable	Agreeable	Nil
9	Normal	Agreeable	Agreeable	Nil
10	Normal	Agreeable	Agreeable	Nil
11	Normal	Agreeable	Agreeable	Nil
12	Normal	Agreeable	Agreeable	Nil
13	Normal	Agreeable	Agreeable	Nil
14	Normal	Agreeable	Agreeable	Nil
15	Normal	Agreeable	Agreeable	Nil
16	Normal	Agreeable	Agreeable	Nil
17	Normal	Agreeable	Agreeable	Nil
18	Normal	Agreeable	Agreeable	Nil
19	Normal	Agreeable	Agreeable	Nil
20	Normal	Agreeable	Agreeable	Nil
21	Normal	Agreeable	Agreeable	Nil
22	Normal	Agreeable	Agreeable	Nil
23	Normal	Agreeable	Agreeable	Nil
24	Normal	Agreeable	Agreeable	Nil
25	Normal	Agreeable	Agreeable	Nil

3.4. Physico Chemical Parameters

TDSs are a measure of the dissolved content of both inorganic and organic substances present in a liquid. The two methods of measuring TDS are gravimetric analysis and conductivity and among these gravimetric methods are the most efficient. The TDS in drinking water comes from normal water sources, sewage, industrial effluents, and chemicals used in the water treatment and the hardware. The TDS concentration is the total cations and anions in the water. Thus, it gives a qualitative measurement of the number of dissolved ions but does not tell us nature or ion relationships.

TDS contents of various types of water brands varied considerably from the standard. All bottled water samples TDS contents were below the standard level (1000 mg/L). Drinking water is normally having some visible solids. These are both organic and inorganic. The drawback of TDS is that they are aesthetically unacceptable. In addition, they have some pathogenic microbes. Further, TDS may also impart palatability, color, and odor to water. Table 2 is indicated the level of pH and TDS in different brands of water samples analyzed in present study.

TDS levels for drinking water varied with different governments. Based on the BSTI guidelines, the higher level of TDS for mineral bottled water and natural drinking water are 1000 mg/L and 500 mg/L, respectively [BSI]. A review was carried out BSI, United States, Canadian, WHO, and European Community (EC) for drinking water standards. The BSI (IS10500), United States, suggested the higher level of TDS is 500 mg/L for drinking water, the Canadian guidelines suggest <1000 mg/L, and the EC Maximum Admissible Concentration (MAC) is 1500 mg/L. None of them has optimum levels of TDS. It can be presumed that based on these guidelines, TDS level can be even 0.00 mg/L in the drinking water which does not have any minerals. Without minerals water could be transparent, clear, and drinkable, but this water will not fulfill the body demand and there will be no taste. Hence, essential minerals need for good taste and to fulfilling the lack of minerals for public health. An isolated report, a summary of Russian studies with the WHO, has suggested that human body fluid and electrolytes are better replaced with water containing a minimum of 100 mg/L of TDS. The US Navy has employed distilled sea water for human consumption for almost 40 years. TDS levels below 3 mg/L utilization of this water for months at a time are common on submarines, no health problems were reported by Navy. The US Army uses reverse osmosis units to supply drinking water for soldiers in the field. The USEPA conducted a project in San Ysidro, New Mexico, in which the TDS was dropped from 800 mg/L to 40 to 70 mg/L, no health effects were recorded. NASA has observed no ill effects from the consumption of almost 0.05 mg/L TDS water on board space craft.

The pH value of various brands of water varies significantly from the standard value (6.5–8.5). The pH of all bottled water samples was within range to standard level (6.5–8.5). At pH above 8.5, mineral incrustations and bitter tastes take place and is also a significant

Table 3: MPN values for 100 ml of sample and confidence limits for various combinations of positive and negative results.

Name of the Sample	No. of Positive Tubes			MPN Index (100 ml)	95% Confidence Limits	
	10 ml	5 ml	0.1 ml		Lower	Upper
1	0	0	0	< 2	< 1	7
2	0	0	0	< 2	< 1	7
3	1	1	0	4	1	11
4	0	0	0	< 2	< 1	7
5	0	0	0	< 2	< 1	7
6	0	0	0	< 2	< 1	7
7	0	0	0	< 2	< 1	7
8	0	0	0	< 2	< 1	7
9	0	0	0	< 2	< 1	7
10	0	0	0	< 2	< 1	7
11	0	0	0	< 2	< 1	7
12	0	0	0	< 2	< 1	7
13	0	0	0	< 2	< 1	7
14	0	0	0	< 2	< 1	7
15	0	0	0	< 2	< 1	7
16	0	0	0	< 2	< 1	7
17	0	0	0	< 2	< 1	7
18	0	0	0	< 2	< 1	7
19	0	0	0	< 2	< 1	7
20	0	0	0	< 2	< 1	7
21	0	0	0	< 2	< 1	7
22	0	0	0	< 2	< 1	7
23	0	0	0	< 2	< 1	7
24	0	0	0	< 2	< 1	7
25	2	1	1	9	2	21

decrease in the efficiency of chlorine disinfection and alum coagulation [42]. In Table 2, the pH values given from 5.9 to 7.8 in the present study. As per the WHO guidelines for drinking water, the pH is in between 6.5 and 8.5. The present results lied between standard levels. In general, the pH is a good indicator to know the water which is hard or soft. In Table 3, all the physicochemical parameters, that is, color, odor, taste, and turbidity for all the screened brands were met the BIS standards.

Drinking water must have a pH of 6.5–8.5 to place within EPA standards, and further note that even within the acceptable pH range, slightly higher or lower pH, and water can be unattractive for various reasons [43]. Higher pH water has a slippery feel, tastes a bit like baking soda, and may depart deposits on fixtures, according to the EPA. Lower pH water may have a bitter or metallic taste and may contribute to fixture corrosion [43]. Wilkes University observed a further problem associated with drinking water and pH: Higher pH water is often hard. They observed that hard water “does not pose a health risk, but can cause aesthetic problems.” Among problems associated with hard water, they list formation of scale on fixtures, a bitter flavor, difficulty getting soaps to lather, and decreased water-heater efficiency. They suggest that water can be softened with ion-exchange water-softening devices [43]. According to a Wilkes University study, the association of pH with atmospheric gases and temperature is the primary reason why water samples should be tested on a regular basis. While the ideal pH level of drinking water should be 6–8.5, the human body maintains pH equilibrium on a constant basis and will not be affected by water consumption. For instance, stomachs have a naturally lower pH of 2 which is a beneficial for food digestion. The present study pH results were observed in between 6.54 and 8.22 and as per scientific reports, results were satisfactory.

4. CONCLUSIONS

The observation of the present study, three water samples tested were showing higher microbial load than the BIS prescribed limits, the management of water plant needs to be disinfected before packing to avoid water-borne diseases. Fecal contamination was also noted in 8% of water samples, which showed that they were not suitable for drinking. Thus, keeping in mind the rising demand of packaged drinking water, it becomes important for the authorities to monitor its quality control and be licensed by concerned authorities, to safeguard consumer’s health. Physicochemical characteristics of all the water samples were lined with the BIS Standards. Although, the present study is preliminary work and needs to be further explored to open up new avenues in clinical applications.

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