

RESEARCH ARTICLE

A MONTHLY EVALUATION OF MICROBIOLOGICAL AND CHEMICAL QUALITY OF BOTTLED DRINKING WATER

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ABSTRACT

Use of bottled water has flourished in Sri Lanka, owing to its high demand, however, the quality of commercially available bottled drinking water is questionable. The objective of the current study was to assess the monthly variation of microbiological and chemical quality of bottled water. Three brands were selected, twenty-four bottles of each brand were collected for analysis. Microbiological and chemical analysis were carried out monthly. Though the shelf life of bottled drinking water is one year, the experiment was terminated in eight months as bacterial counts showed a steady decline with time as follows. The results indicated that Total Coliform [TC], Fecal Coliform [FC] and Heterotrophic Plate Count [HPC] bacteria decreased throughout the shelf life. There were significant differences in TC ($P<0.05$) and FC ($P<0.01$) between microorganisms initially present and after eight months of storage. Even though SLSI permitted levels for presumptive TC is less than zero cfu per 100ml, the average count of TC was 139 cfu per 100 ml at the end of the first month. According to national and international standards, the FC count should be zero per 100 ml for drinking water. However, at the first month some bottled water samples exceeded this limit for presumptive FC, with an average count of 32.5 cfu per 100 ml. For HPC bacteria, only one brand exceeded the WHO guidelines (50 cfu/ml). No algal species were detected. Fungal colonies showed a reduction in number over an eight-month duration. *Penicillium* sp. and *Aspergillus* sp. were dominant. Chemical parameters were within the permitted levels, except hardness in water samples. The result of this study reveals that the bottled water industry needs to be monitored closely and continuously by relevant authorities, intending to provide safe bottled water to the public.

Keywords: *Bottled water, Microbiological and chemical quality, Monthly variation*

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1. INTRODUCTION

Bottled water can be defined as any potable water that is bottled and distributed or offered for sale and specifically intended for human consumption. Sales of bottled water are increasing for many reasons. In many developing countries, tap water is contaminated or thought to be contaminated with various forms of pollutants. In regions, where reliable, low-cost, and high quality tap water is not available, bottled water provides a safe alternative. Bottled water is regulated as a 'food' by the US Food and Drug Administration (FDA). The FDA requires that bottled water to be clean and safe for human consumption, that they are processed and distributed under sanitary conditions and they are produced in compliance with FDA good manufacturing practices (https://www.fda.gov/food/buy-store-serve-safe-food/fda-regulates-safety-bottled-water beverages-including-flavored-water-and-nutrient-added-water).In applying the guidelines to bottled waters, certain chemical constituents and microbial contents may be more readily controlled than in tap water. Some microorganisms that are normally of little or no public health significance may grow to higher levels in bottled water [1].

The bottled water industry has become a vital and vigorous sector in developed and developing countries worldwide. In Europe, The United States, North America, Canada and in other developed countries bottled water is one of the most important food items of high demand. As a result, bottled water consumption has significantly increased [2, 3, 4, 5, 6]. Further, the consumption of bottled water in Iran has increased particularly among the urban population and travelers [7], as there is a general belief among consumers that bottled water is safe and free of all impurities, including bacteria [8]. This reflects consumer concerns about tap water, since bottled water is often regarded as safer as and healthier than tap water.

However, microbial surveys carried out worldwide indicated various problems with bottled water such as high Heterotrophic Plate Count (HPC) levels [2, 9], *Vibrio cholerae* presence and infections [10], fungal spoilage [11], *Pseudomonas aeruginosa* [3, 12]. Therefore, the bottled water industry has to exhibit strict quality standards in terms of microbial parameters, production processing, bottling, transportation and storage [13, 14], while Hazard Analysis Critical Control Points (HACCP) systems should be implemented in the bottling process [3].

In Sri Lanka use of bottled water has been increasing over the last two decades. However, due to the increased demand and consumption of bottled water in Sri Lanka, there has been a growing concern about the microbiological quality of this product. Several studies done in Sri Lanka concerning the quality of the bottled water have reported that they have exceeded the levels permitted by the Sri Lankan Standard Institution for the presumptive total coliforms [15, 16]. With this background, the main objective of this study was to assess the monthly variation of microbiological and chemical quality of bottled water during storage.

2. MATERIAL AND METHODS

2.1 Sample Collection

To determine the microbiological and chemical quality of bottled water three bottled water brands were collected. Twenty-four bottles of each brand were collected for analysis. All bottles were collected from the Kandy district, of the central province of Sri Lanka. The samples were stored at room temperature (27±2 ºC) and analyzed monthly after the date of manufacture throughout the first eight months of the shelf life of the bottled water, while the shelf life of bottled water is one year. (The experiment was terminated after 8 months as bacterial counts showed a steady decline and FC counts reduced to zero as early as in the 2nd month.)

2.2 Microbiological analysis

Total and fecal coliforms were counted by membrane filtration method [17] passing 100 ml volumes of each sample through the membrane filtration apparatus (Pyrex, Germany) using sterilized membrane filters (Sartorius, Germany) with 0.45 µm pore sizes. Membrane filters were placed on pre sterilized absorbent pads (Sartorius, Germany), saturated with 3 ml of M-Endo broth (HI-media, India) (for TC) and 3 ml of M-FC broth base (HI-media, India) (for FC). Plates were incubated for 24-48 hours at 36 ± 1 °C and at 44.5 °C for the detection of total coliforms and fecal coliforms respectively. Sterilized distilled water and typical coliforms (*Serratia marcescens* -NCTC 11935, *Escherichia coli* **-**ATCC 25922) were used as a negative control and a positive control respectively in detection of coliform bacteria. Typical red color colonies with a green metallic sheen were counted as total coliforms on M-Endo medium. In addition, red- pink colonies with a sheen were counted. Typical blue color colonies formed on M-FC medium were counted as fecal coliforms. Numbers of yellow colour atypical colonies on M-FC were also recorded. Heterotrophic Plate Count (HPC) bacteria present in bottled water samples were determined by the pour plate method [18]. The number of bacterial colonies was reported as colony-forming units per milliliter (cfu/ml). Analyses were carried out in triplicate for all determinations. Fungi were isolated by spreading 0.1 ml aliquots of bottled water samples on Potato Dextrose Agar plates, and the plates were incubated at room temperature for 4-5 days. Identification was done by observing colony characteristics, reproductive morphology through microscopic observations and with the aid of reference materials [19, 20]. Algae in bottled water samples were estimated in a zigzag pattern using a Sedgwick rafter cell, under a light microscope.

Confirmation of presumptive coliform bacteria

Selected colonies isolated on M-Endo and M-FC media were confirmed for total coliforms and fecal coliforms respectively. Presumptive total and fecal coliforms colonies were sub cultured by streaking on Tryptone Soy Agar (TSA) (OXOID-

CM0131). Subsequently, well isolated colonies from TSA plates were subjected to confirmation. Total coliforms and fecal coliforms were confirmed by inoculating colonies into Brilliant Green Lactose Broth (BGLB) (HI-media, India) tubes and peptone water tubes respectively.

2.3 Chemical analysis

Physiochemical parameters were analyzed following the standard guidelines and procedures [21]. The alkalinity, hardness and the chloride (Cl^-) contents were determined by titration methods using Hach digital titrator and Hach standard reagent cartridges. Calcium (Ca), iron (Fe), manganese (Mn) and zinc (Zn) were measured by the atomic absorption spectrometer (Varian 240FS Inc., Australia) and spectrophotometer (Hach DR-2400 with standard reagents) was used to determine nitrate (NO^{3-}), nitrite (NO^{2-}), phosphate (PO_4^{3-}), fluoride (F^-), ammonium (NH_4^+), sulphate (SO_4^{2-}) and sulfide (S^{2-}). All instruments were calibrated using commercially available standard solutions (BDH, Fulka) before performing the measurements.

3. RESULTS AND DISCUSSION

Over the shelf life of the bottled water samples, a reduction in number of colony forming units were observed. Table 1 shows the analysis of variance applied to microbiological quality of bottled water samples. For all three bacteriological parameters (TC, FC, HPC bacteria), bacterial counts decreased with time during the eight months of the shelf life of bottled water. There were significant differences ($P < 0.05$) in total coliforms numbers between the numbers of microorganisms initially present, with an average count of 139 cfu per 100 ml at one month in the water and those present after eight months of storage, which decreased to 4 cfu per 100 ml, while the level permitted by SLSI for TC is zero cfu per 100 ml [22]. According to national and international standards, the FC count should be zero per 100 ml for drinking water. However, at the first month some bottled water samples exceeded this limit for presumptive fecal coliforms, with an average count of 32.5 cfu per 100 ml, which declined to zero cfu per 100 mL at eight months, exhibiting a significant difference ($P < 0.01$) in the number of presumptive FC between one month and eight months after storage (Table 1). This reduction may be due to nutrients depletion during storage. Fecal coliforms were absent in all bottles of one brand analyzed in the study.

Coliform bacteria are used as an indicator organism in assessing drinking water quality. Coliforms constitute a large portion of human intestinal microflora. These ensure the presence of indicator organisms providing evidence for contamination of water with human faeces. However, there are some coliforms found in plant and soil samples. Therefore, the numbers of TC should be higher than FC, generally present as fecal contaminants. Presence of FC in water is considered as an accurate indication of contamination from fecal matter rather than presence of TC [23]. Coliform organisms have long been recognized as a good microbiological indicator of drinking water quality, due to their ease of detection and quantification in water [1].

Table 1: Analysis of variance (ANOVA) of microbial quality of bottled water stored during 8 months after the date of manufacture. (the data were converted to arcsin values to minimize gaps [Zar, 1999]); Mean values followed by the same superscript (a & b) within a column, do not differ significantly ($p<0.05$) for TC, ($p<0.01$) for FC and ($p<0.05$) for HPC

Time Period (months)	Mean of presumptive TC cfu per 100 ml	Mean of presumptive FC cfu per 100 ml	Mean of HPC cfu per ml
1	139.00^{a}	32.50^{a}	84.00^{a}
2	42.83^{b}	0.00^{b}	60.50^{a}
3	23.33^{b}	0.50^{b}	38.67^{a}
4	15.00^{b}	0.00^{b}	30.83^{a}
5	11.33^{b}	0.00^{b}	19.83^{a}
6	7.33^{b}	0.00^{b}	12.83^{a}
7	7.33^{b}	0.00^{b}	11.83^{a}
8	4.00^{b}	0.00^{b}	8.33^{a}

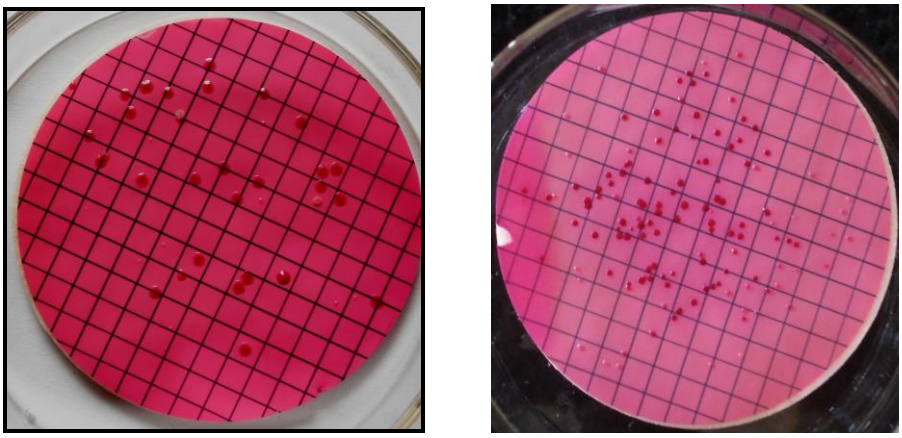
Several studies done in different countries have revealed that bottled water were inappropriate for human consumption due to the presence of different microorganisms. A study done in Nepal stated that out of 100 samples, 48% of samples were found to be contaminated with total coliform such as, *E. coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* [24]. Another study done in Nepal has shown bottled water brands commercially available were found to be contaminated with indicator organisms in drinking water and the counts exceeded WHO standards [25]. Further, a study done in Mexico stated that total coliforms, fecal coliforms, and *E. coli* were found in bottled water samples tested and certain water samples exceeded the maximum allowable limit imposed by Mexico's standard guidelines [26]. Moreover, many studies done in Sri Lanka stated that bottled water brands tested exceeded permitted level for microbiological parameters [9, 10, 16, 27].

It is important to conduct confirmation test for presumptive coliform in assessing drinking water quality as confirmation. In the current study twenty percent of presumptive total coliform and fecal coliform colonies were used for confirmation testing. With respect to total coliforms confirmation was done for typical red colonies with a green metallic sheen and for red-pink colonies with a sheen (Figure 1). With respect to fecal coliforms confirmation was done for typical blue colonies and atypical green/yellow colonies (Figure 2). During the current study, typical red colonies with a green metallic sheen on M-Endo medium were confirmed as total coliforms while red/

pink colour colonies with a sheen were not confirmed as total coliforms. Furthermore, certain typical blue colonies on M-FC medium were confirmed as fecal coliforms (Table 2). However, according to [28] both pink colour colonies and atypical cream colour colonies (total of colonies isolated from well water, river water, effluent water and bottled water) isolated on M-Endo medium have been confirmed as total coliforms ranging from a confirmation rate of 55 % – 88 %. The difference of these confirmation results may probably be due to the fact that, bottled water being subjected to UV radiation before bottling, altering the cell characteristics of these pathogens and not showing a typical reaction in selective media or during the confirmation. This also raises the question of the pathogenicity of these organisms. This hypothesis will have to be examined further.

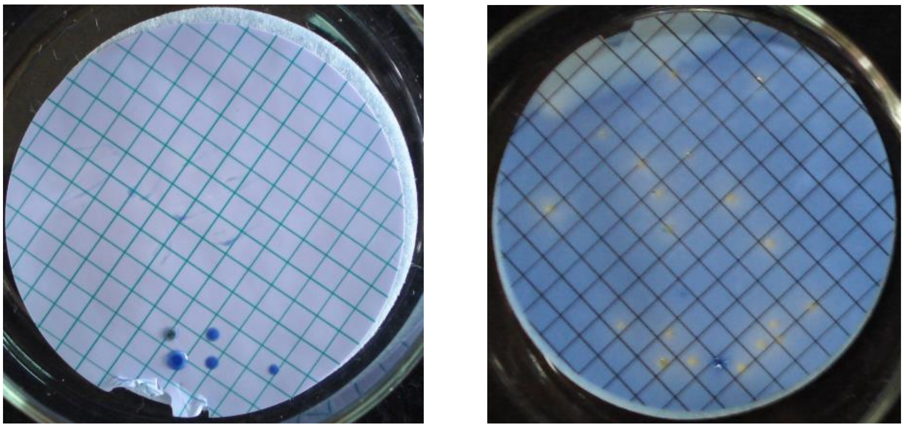
During storage, HPC bacteria was detected in all three brands tested, although only one brand exceeded the permitted level for drinking bottled water. According to the results, during the eight months of the shelf life of bottled water samples, they showed a reduction in number of colony forming units for HPC bacteria (Table 1). The presence of high number of heterotrophic bacteria in bottled water is probably due to microorganisms naturally occurring in source water.

Many studies done in different countries have revealed that bottled water may contain a range of microorganisms, including *Aeromonas, Alcaligenes, Arthrobacter, Caulobacter, Corynebacterium, Flavobacterium,* and *Pseudomonas* [29, 30, 31, 32]. It is not reported that these HPC bacteria represent a health affect through water consumption by the general public. However, severely immunocompromised persons are not recommended to consume drinking water contaminated with these microorganisms.



(a) (b)

Figure 1: Presumptive total coliforms species on a M-Endo plates with typical red colonies with a green metallic sheen (a) and red-pink colonies with sheen (b)



(a) (b)

Figure 2 : Presumptive fecal coliforms species on a M-FC plates with typical blue colonies (a) and atypical colonies (yellow colour) (b)

Table 2: Confirmation test results for detecting presumptive total and fecal coliforms

	Number of colonies used for confirmation	Number confirmed by testing	Confirmation rate %
Total coliforms			
Typical red colour with a green metallic sheen colonies	23	23	100
Red/pink colour colonies with a sheen	35	0	0
Fecal coliforms			
Typical blue colonies	15	15	100
Atypical yellow colonies	10	0	0

During the current study, no algal species were detected in any of the tested bottled water samples throughout the eight-month duration. All the water samples examined were contaminated with fungal species. Among the fungal species isolated, *Penicillium* sp. and *Aspergillus* sp. were dominant while, *Cladosporium* sp. and *Trichoderma* sp. were detected in lesser numbers. In considering the result of fungal colonies, a reduction in number over the eight-month duration was observed. Some of the fungi isolated from bottled water samples are species commonly found in the environment, while some of these fungi can cause diseases in humans. eg; chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis [33]. *Alternaria alternata* and *Penicillium citrinum* have some toxigenic potential and could constitute some health risk. It is therefore advisable to assess fungal propagules in routine microbiological studies of bottled drinking water to establish baselines [34].

Chemical parameters are important factors of drinking water. Drinking water may contain several minerals (e.g., fluoride, potassium, zinc) and trace constituents (e.g., zinc, arsenic, manganese. iron, cyanide, lead) that are associated with both benefits and risks for public health [4].

Table 3: Summary of results of chemical quality of bottled water analysis during 1st and 8th months after manufacturing. Parameter concentrations are given in mg/l unless otherwise specified. SD – Standard Deviation

Parameter	Initial (1st month)				Final (8th month)				Permitted level
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	
pH	5.75	6.8	6.37	0.54	5.32	6.89	6.22	0.81	6.5 to 8.5
EC (µS/cm)	15	146	83.33	65.68	17	148	85	65.64	2500
Alkalinity	4	14	9.73	5.16	5.6	60	36.53	27.96	200.0
Hardness	9.2	34.8	24.93	13.37	8.8	48.8	33.73	21.75	10-20
Chloride	7.5	22.5	13	8.05	11.25	39.5	21.17	15.89	250.0
Fluoride	0	0.35	0.23	0.2	0.12	0.21	0.17	0.05	1.5
Sulfate	0	4	1.67	2.08	1	1	1	0	250.0
Sulfide (µg/l)	0	32	11	18.19	0	0	0	0	50
Phosphate	0.01	0.12	0.06	0.06	0.12	0.19	0.16	0.04	5
Nitrate-N	2.7	3.6	3.3	0.49	0.3	0.6	0.47	0.15	50.0
Nitrite-N	0.003	0.007	0.005	0.002	0.005	0.007	0.006	0.001	3.0
Ammonium-N	0	0.04	0.01	0.02	0	0.02	0.007	0.011	0.5
Zn	0	0.1	0.003	0.006	0	0.04	0.01	0.02	3.0
Ca	0.49	5.12	3.32	2.482	0.11	4.85	2.87	2.46	150
Mn	0	0.1	0.007	0.006	0.01	0.03	0.02	0.01	0.05
Fe	0	0.1	0.007	0.006	0.01	0.03	0.02	0.01	0.2

Chemical analysis of bottled water samples collected showed that most of the parameters investigated slightly deviated from the permitted levels of WHO and SLS at the first month and at the 8th month.

In the present study, the minimum and maximum values of pH in bottled water were 5.32 and 6.89 respectively. The minimum value was slightly below the WHO and SLS permitted levels. At the beginning and the end of the analysis maximum values of hardness in water samples exceeded the permitted levels. Values of alkalinity and electric conductivity did not exceed the permitted levels recommended by the Health Ministry, Sri Lanka, and the values were very low (Table 3). However, NO_2^-, Cl^- and PO_4^{3-} levels were higher at the 8th month than that of the initial values for most of the samples, though within the permitted levels. NO_3^-, SO_4^{2-}, S^{2-}, NH_4^+, F and Ca levels were higher at the beginning and decreased with time and were all below their respective permitted levels.

According to the information given on the labels of bottled water, most of the source water for bottling in Sri Lanka, is from the central hill regions or wet zone of the country, where lower levels of fluoride is observed in water compared to that of water from the dry zone. In bottled water samples tested in the current study, the maximum fluoride level was 0.35 mg/l, which does not have any detrimental health effects accordingly to WHO guidelines.

CONCLUSION

The results of the current study indicate concerns over the bottled water industry in Sri Lanka, according to the results, some bottled water samples were not safe for drinking according to the Health Ministry regulation in Sri Lanka (The Gazette of the Democratic Socialist Republic of Sri Lanka, 1420/4, 21/11/2005) as they exceeded the permitted levels for one or more of the microbial parameters analyzed. When considering the tested chemical parameters, the chemical quality of bottled water was within permitted levels, except hardness in water samples. It is also revealed that both bacteriological and chemical parameters can change during the storage of bottles. Therefore, bottled water industries need to be closely and continuously monitored by relevant authorities, intending to provide safe bottled water to the public.

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