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SHORT COMMUNICATION

ASSESSMENT OF MICROBIOLOGICAL AND CHEMICAL QUALITY OF FIVE LITER VOLUME BOTTLED DRINKING WATER

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ABSTRACT

Consumption of bottled drinking water has dramatically increased among the community in recent years worldwide and also in Sri Lanka. The objective of this study was to assess microbiological and chemical quality of five liter volume bottled water during the shelf life period. Three five liter bottles each of three brands were collected for analysis. Microbiological and chemical analyses were carried out monthly after the date of manufacture throughout the first three months of the shelf life of the bottled water. The results indicated that Total Coliform [TC] and Faecal Coliform [FC] counts decreased with time over the first three months of shelf life. There were no significant differences ($P > 0.05$) in TC between the numbers of microorganisms initially present, with an average count of 107.19 cfu per 100 ml and those present after three months of storage, which decreased to 33.70 cfu per 100 ml, while the level permitted by SLSI for TC is zero cfu per 100 ml. According to national and international standards, the FC count should be zero per 100 ml for drinking water. However, bottled water samples tested exceeded this limit for presumptive FC, with an average count of 0.63 cfu per 100 ml, which declined to 0.3 cfu per 100 ml after three months, without exhibiting a significant difference ($P > 0.05$). Chemical parameters were within the permitted levels, except pH and hardness in water samples. Therefore, bottled water available for sale needs to be monitored continuously by relevant authorities, in order to provide safe bottled drinking water to consumers.

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

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1. INTRODUCTION

Consumption of bottled drinking water has dramatically increased among the community in recent years worldwide and also in Sri Lanka, and this trend is expected to continue. Individuals prefer to use bottled water in emergency situations or when the taste and odor

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of other available drinking water are undesirable [1, 2, 3, 4]. Bottled water is also consumed by a wide range of people, including the elderly, children and pregnant women. Therefore, the safety of bottled water must always be assured. Even though the public opinion is that bottled water is considered completely suitable to drink, bottled water can sometimes be contaminated by physicochemical and biological agents [5, 6, 7, 8]. Therefore, concern about the bottled water quality has been rising due to the significant increase in bottled water consumption over the last decade. However, several studies on the quality of bottled water reported violations of some drinking water permissible limits [9, 10, 11, 12, 13, 14]. Several studies have documented the detection of coliforms and heterotrophic bacteria in bottled water in counts which far exceeded national and international standards set for potable water for human consumption [9, 10, 11, 12, 13, 14]. It is reported that bottled water is not sterile as it may contain various pathogens like coliforms, *Escherichia coli*, *Pseudomonas* spp., Campylobacter or even mycobacteria [15, 16, 17, 10, 11, 12]. Furthermore, most epidemiological studies have historically reported the occurrence of diseases, including problems with reproduction, cancer, congenital malformations of the central nervous system, cardiovascular diseases and even death due to exposure to trace elements and mineral contents of water [18, 19, 20, 5]. As a result, there may be considerable risk to humans, especially children exposed to bottled water containing microbiological entities and toxic elements.

When considering the source water, it may be from springs, dug wells, tube wells or municipal systems, which are considered to be safe and of good sanitary quality and fit for human consumption. However, it is also the case that water from springs, wells and municipal supplies are known vehicles for enteric pathogens and water quality is often related to the degree of bacterial contamination [21, 22]. The presence of these microorganisms can have an impact on human health.

With the increased demand and consumption of bottled water in Sri Lanka, there has been a growing concern about the microbiological and chemical quality of these products. Though SLS has stipulated standards (SLS 614 - 1983, SLS 894 – 2003), there are doubts as to whether these standards are adhered to and research has not been carried out adequately on the microbial and chemical quality, especially five L volume bottles, while bottling industries are mushrooming all over the country. With this background, this study was conducted to assess the microbiological and chemical quality of five L volume bottled water during the shelf life period.

2. METHODS AND MATERIALS

2.1 Sample collection

Three brands of bottled water, of which 500 ml bottles were previously assessed and regarded as contaminated, were used in this study [13]. Three five L bottles of each brand consisting of the same batch number were collected. All bottles were collected from the Kandy district, Sri Lanka. The samples were stored at room temperature (27±2 ºC) and analyzed monthly after the date of manufacture throughout the first three months of the shelf life of the bottled water.

2.2 Microbiological analysis

Total and fecal coliforms were enumerated by membrane filtration method [23], 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. Subsequently, the membrane filters were placed on presterilized absorbent pads (Sartorius, Germany), saturated with 2.5 ml of m-ColiBlue24 medium (Hach). Plates were incubated for 24-48 hours at 36 ± 1 °C for the detection of total coliforms (TC) and fecal coliforms (FC). Sterilized distilled water and typical coliforms (*Serratia marcescens* -NCTC 11935, *Escherichia coli* -ATCC 25922) were used as a negative control and positive control, respectively in the detection of coliform bacteria. Typical red colonies and blue colonies on the membranes were counted and reordered. Total coliforms were enumerated as a total of red and blue colonies, while blue colonies were taken as fecal coliforms. Analyses were carried out in triplicate.

2.2.1 Statistical evaluation

The following null hypothesis was examined; there is no significant difference between the numbers of microorganisms initially present in bottled water and those present after three months of storage at ambient temperature. The data were converted to arcsin values to minimize the gaps and the analysis of variance (ANOVA) was performed to check the difference in the population of microorganisms at different time periods of storage.

2.3 Chemical analysis

Physiochemical parameters were analyzed following the standard guidelines and procedures [24]. The alkalinity, hardness and 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

Every country has its own drinking water standards that prescribe which substances can be in drinking water and their maximum concentrations. The standards are called maximum contaminant levels. They are formulated for any contaminant (whether a substance or a foreign bacterial, viral or fungal element or a chemical substance) that may have adverse effects on human health. Each company that prepares drinking water has to follow the stipulated standards. The Ministry of Health and Sri Lankan Standards Institute (SLSI) have stipulated the standards for drinking water in Sri Lanka.

Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water [25]. In drinking water from municipal supplies, the coliform test can be used as an indicator of treatment efficiency and of the integrity of the distribution system. Although coliform organisms may not always be directly related to the presence of fecal contamination, the presence of coliforms in drinking water suggests the potential presence of pathogenic enteric microorganisms such as *Salmonella* spp., *Shigella* spp., and *Vibrio cholera,* which could give rise to human diseases.

In the current study, over the first three months of shelf life of the bottled water samples, a reduction in number of colony forming units was observed. Table 1 shows the analysis of variance applied to microbiological quality of bottled water samples. For all two bacteriological parameters (TC and FC), bacterial counts decreased with time during the three months of the shelf life of bottled water. There were no significant differences ($P > 0.05$) in TC numbers between the numbers of microorganisms initially present, with an average count of 107.19 cfu per 100 ml at the first month and those present after three months of storage, which decreased to 33.70 cfu per 100 ml, while the level permitted by SLSI for TC is zero cfu per 100 ml [26]. According to national and international standards, the FC count should be zero per 100 ml for drinking water. However, bottled water samples tested exceeded this limit for presumptive fecal coliforms, with an average count of 0.63 cfu per 100 ml, which declined to 0.3 cfu per 100 ml at three months, without exhibiting a significant difference ($P > 0.05$) in the number of presumptive FC between one month and three months after storage (Table 1). The brands tested in the current study, throughout the first three month of shelf life of the bottled water, total and fecal coliforms colonies decreased with time. The number of colony forming units was high at the beginning and then decreased thereafter. Similarly, [27, 10, 13] have noticed that after storage of bottled drinking water at room temperature (18–25 ^{o}C), an overall decrease in the number of microbes recovered. The reason for the decrease in microbial counts with a long period of time could be the exhaustion of nutrients and competition by microbes in bottled water during storage. However, [15] has noted that the viable counts of bacteria increases notably in bottles after 1-3 weeks of storage.

Table 1: Analysis of variance (ANOVA) of microbial quality of bottled water (5 L volume) stored during 3 months after the date of manufacture.

Time Period (months)	Mean of presumptive TC cfu per 100 ml	Mean of presumptive FC cfu per 100 ml
1	107.19^{a}	0.63^{b}
2	95.44^{a}	0.52^{b}
3	33.70^{a}	0.30^{b}

The data were converted to arcsin values to minimize gaps [28] Mean values followed by the same superscript (a & b) within a column, do not differ significantly ($p<0.05$) for TC, ($p<0.05$) for FC. Further, a similar study conducted by (29) stated that TC and heterotrophic bacteria counts in 20 L and 5 L bottled water, available at the Rajarata University of Sri Lanka exceeded the permissible levels.

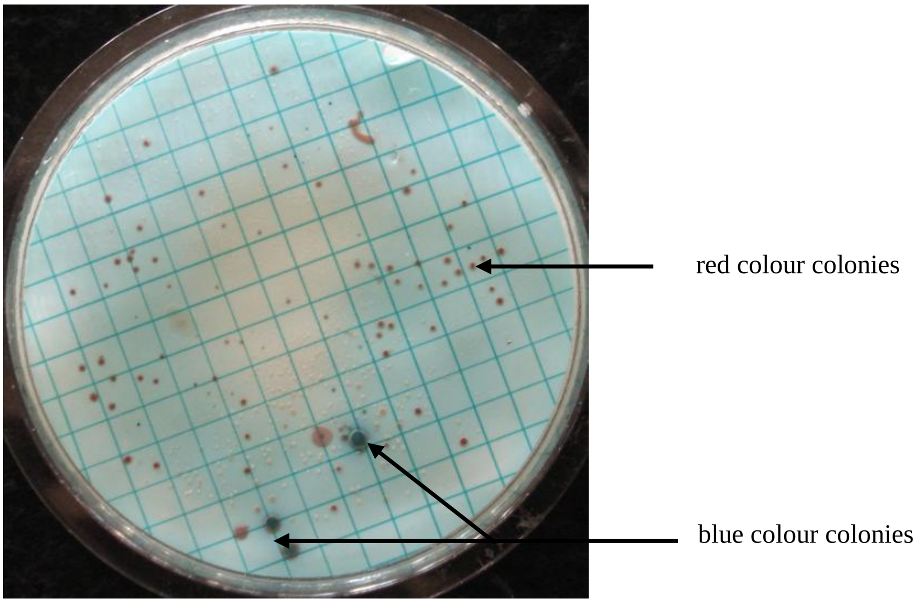

Figure 1: m-ColiBlue24 plate with presumptive total and fecal coliforms colonies

Chemical contaminants in drinking water pose health risks for infants, the elderly, and people with weakened immune systems due to viral infections, immune disorders, cancer, chemotherapy, or recent organ transplantation [25]. Chemical analysis of bottled water samples collected showed that most of the parameters were within the permitted levels of WHO and SLS at the initial measurements as well as measurements at the end of the 3 months period (Table 2).

In the present study, the minimum and maximum values of pH in bottled water were 6.11 and 6.72 respectively. The minimum value was slightly below the WHO and SLS permitted levels. However, F, Mn and Fe contents were higher at the end of 3 months period than that of the initial values for most of the samples. However, all were within the permitted levels for the said parameters. NO_3^-, NO_2^-, NH_4^+, SO_4^{2-}, S^{-2}, Cl^-, PO_4^{3-} and Ca contents were higher at the beginning but decreased with time in most of the samples and all parameters were below their respective quality guidelines. Values for alkalinity and electric conductivity were within permitted levels recommended by the Health Ministry except pH, while the values were below the permitted level for some brands and hardness, while exceeding the permitted level.

Table 2: Summary of results of chemical quality of bottled water analysis during 1st and 3rd month after manufacturing. Parameter concentrations are given in mg/l unless otherwise specified

Parameter	After 1 month				After 3 months				Permitted
	Min	Max	Mean	SD	Min	Max	Mean	SD	level
pH	6.11*	6.47	6.29	0.12	6.37*	6.72	6.62	0.12	6.5 to 8.5
EC (µS/cm)	99	184	135.44	37.17	96	182	134.89	37.55	2500
Alkalinity	14.3	50	30.53	14.15	26.8	56.3	39.01	12.36	200.0
Hardness	35.6	59.6	46.44	10.22	36	63.6	47.56	11.93	10-20
Chloride	12.5	39.75	23.78	11.39	10.75	39.5	24.78	12.7	250.0
Fluoride	nd	0.18	0.04	0.07	nd	0.7	0.12	0.23	1.5
Sulfate	3	42	15.44	12.61	nd	16	6.78	6.91	250.0
Sulfide (µg/l)	nd	14	5.56	5.39	1	12	4.11	3.26	50
Phosphate	nd	0.18	0.09	0.06	nd	0.14	0.06	0.05	5
Nitrate-N	2.7	3.6	3.3	0.49	0.3	3.2	1.2	1.1	50.0
Nitrite-N	0.004	0.032	0.011	0.01	0.004	0.007	0.005	0.001	3.0
Ammonium-N	nd	0.05	0.02	0.02	nd	0.02	0.002	0.007	0.5
Zn	nd	0.01	0.002	0.004	nd	0.01	0.004	0.005	3.0
Ca	2.38	6.44	4.53	1.62	1.02	5.93	3.01	2.17	150
Mn	nd	0.01	0.002	0.004	nd	0.03	0.006	0.01	0.05
Fe	nd	0.02	0.008	0.007	0.01	0.04	0.018	0.011	0.2

(n=3; * below the permitted level; SD – standard deviation; nd – not detected)

CONCLUSION

The results of this study show concern over the bottled water industry in Sri Lanka. According to the results, bottled water brands were inappropriate for drinking in accordance with the Health Ministry regulation in Sri Lanka as they exceeded the permitted levels for TC and FC. When considering the tested chemical parameters, the chemical quality of bottled water was within permitted levels, except pH and hardness in water samples. Therefore, bottled water available for sale needs to be monitored continuously by relevant authorities, in order to provide safe bottled drinking water to consumers.

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