Evaluation of microbiological quality of commercially available bottled drinking water in Colombo district, Sri Lanka


Highlights
- Of all bottled water brands tested, 50%, 24% and 35% have violated WHO, SLSI, and the Sri Lanka Health Ministry permissible levels for total coliforms, faecal coliforms and heterotrophic plate counts (HPC), respectively.
- *Aspergillus* spp., *Rhizopus* sp., *Trichoderma* sp. and *Mucor* sp. were the dominant fungi, while 8% were positive for *Chlorella vulgaris*.
- The HPC and algal counts were notably higher in tube wells than that of dug wells and natural springs.
- This industry needs a competent regulatory body to ensure safe bottled drinking water for consumers.
Evaluation of microbiological quality of commercially available bottled drinking water in Colombo district, Sri Lanka

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Abstract: In recent times, the consumption of bottled water has dramatically increased in Sri Lanka. However, compliance by the producers with the bottled water regulations is debatable, which poses questions about bottled water quality. This study aimed at evaluating the microbiological quality of bottled water in the Colombo district, Sri Lanka. Twenty-six brands of drinking water were collected from the Colombo district (19 locations) microbial quality was detected by checking the total coliforms (TC), fecal coliforms (FC), heterotrophic bacteria, fungi and algae. The results revealed that 50 % of drinking water brands violated the Sri Lanka Standards Institution (SLSI) and WHO guidelines, and the Sri Lanka Health Ministry regulation (0 cfu/100 ml). Twenty-three percent of brands exceeded the limits for presumptive FC (0 cfu/100 ml in accordance with WHO guidelines, SLSI, and the Sri Lanka Health Ministry requirement). Moreover, 35% showed higher heterotrophic bacteria which exceeded the WHO guidelines (50 cfu/ml). The dominant fungi found in the bottled water were Aspergillus spp., Rhizopus sp., Trichoderma sp. and Mucor sp. Chlorella vulgaris was identified as the algal species that was present in the drinking water samples and it was 8 % of the samples checked. Additionally, the statistical analysis of water sources revealed no significant differences in the levels of fecal and total coliforms in samples across springs, tube wells, and dug wells. However, the tube wells have a significant difference in HPC and algae than dug wells and springs. The findings of this study concluded that the bottled water industry needs to be closely supervised by competent authorities to provide customers with more healthy bottled water in Sri Lanka.

Keywords: bottled water; microbiological quality; total coliforms; fecal coliforms; heterotrophic bacteria

INTRODUCTION

Bottled water is currently known as a global billion-dollar business and human consumption of bottled water has largely increased due to successful promotions done by the water bottle manufacturers highlighting bottled water characteristics including their safety, purity, and cleanliness (Bharath et al., 2003). Many types of bottled water such as mineral water, purified water, sparkling water, sparkling mineral water, flavored water, near water, and functional water are available all over the world (Rani et al., 2012).

The bottled water concept was established in Sri Lanka in the late 1980s (Sevigny, 2017). Although new brands of bottled water are introduced to the market constantly, it is still questionable whether these water products are safe for drinking. However, local demand for bottled drinking water has drastically increased due to some reasons such as the tourism industry development, natural disasters, health problems due to unsafe drinking water, improved living standards of people, increasing surface and groundwater pollution, branding, and terrorist activities. Bottled drinking water is believed a healthy alternative to tap water, thus leading to the flourishing of this industry in the country (Piyarathna et al., 2020). According to the list of the Food Control Administration Unit of the Ministry of Health, Sri Lanka 2019, there are 178 local bottled water brands and 3 imported brands (from France, Japan, and Italy) are available in Sri Lanka. Further, in Sri Lanka, it is essential to certify the manufacturing or importing process of bottled water by the Sri Lanka Standards Institution (SLSI). To obtain the SLS certificate, the water source must be set up as a spring, tube well, or dug well. However, bottled water brands are available in the Sri Lankan market without SLS standards, quality packaging, and standard water quality information (Piyarathna et al., 2020).

The concern about the microbiological quality of bottled water has grown over the years (Warburton et al., 1986; Bedada et al., 2018; Gautam, 2021). Several studies have documented the detection of coliforms and heterotrophic bacteria in bottled water in counts that far exceeded national and international standards set for potable water for human consumption. It has been reported that bottled water is not sterile as it may contain various pathogens such as Escherichia coli or other coliforms, Pseudomonas spp., Campylobacter, or even Mycobacterium (Bharath et al., 2003; da Silva et al., 2008; Herath et al., 2012; Herath and Abayasekara, 2021). Bottled water has also been the vehicle for the transmission of disease-causing microorganisms such as Vibrio cholerae and Staphylococcus aureus (Leclerc et al., 1985). In Mexican research, total coliforms (TC) and fecal coliforms (FC) were detected and exceeded Mexico’s Official Guidelines (Cerna-Cortes et al., 2019). Another study carried out in Ethiopia found thermotolerant coliforms, Escherichia coli, and Staphylococcus aureus in the bottled water samples. It revealed that about 40 % of

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bottled water samples were bacteriologically unsafe for human consumption (Bedada et al., 2018).

Further, some studies done in Sri Lanka regarding the quality of bottled water have recorded that they have exceeded the levels permitted by the Sri Lankan Standard Institution for the presumptive total coliforms (Herath et al., 2012). Further, Herath et al., in 2014 revealed that there was *Pseudomonas aeruginosa* in bottled drinking water. Another study done in Jaffna Peninsula showed that the bottled water contained *Escherichia coli* and *Klebsiella* spp. and some fungal contaminants (Sasikaran et al., 2012).

Though the bottled water industry has improved worldwide, strict regulations have been established in many countries to manipulate the quality of bottled water in many countries. General guidelines for the quality of water used for human consumption have been recommended by the World Health Organization (WHO) and the European Economic Community (EEC). *Escherichia coli* and thermotolerant coliform bacteria should be zero in any 100 ml of drinking water sample and Heterotrophic Plate Count (HPC) limits of 50 colony-forming units per ml (cfu/ml) of drinking bottled water (WHO, 2011). According to Sri Lankan Standards (Sri Lanka Standards 894: Part 2: 2020) and the Health Ministry regulation in Sri Lanka (The Gazette of the Democratic Socialist Republic of Sri Lanka, 1420/4, 21/11/2005), the TC and FC counts should be zero per 100 ml and Aerobic Plate Count (APC) should be less than 1 x 103 CFU (Colony Forming Unit) per 1 ml of bottled water.

Considering the urbanization rates in Sri Lanka, Colombo is one of the most highly urbanized cities. More than 10% of the population of Sri Lanka lives in the Colombo district. Eighty percent of the water demand in Colombo is fulfilled by the Kelani River and it is a primary source of drinking water (Liyanage and Yamada, 2017). According to the Food Control Administration Unit of the Ministry of Health (2019), twenty-six brands of bottled water are available in the Colombo district markets. The bottled water manufacturers in Sri Lanka mostly use dug wells, tube wells, and springs as a water source for bottling and most bottled water contains purified underground water (Piyarathna et al., 2020). Some of those bottled water manufacturers use underground water from the Kelani River basin to fill the bottled water. The groundwater in the Kelani River basin is poor for drinking purposes and according to the observations, the reason is the inappropriate construction of toilets nearby the river basin causing fecal coliform contamination of the groundwater (Mahagamage et al., 2015). Further, the bacteriological and chemical parameters of the bottled water could be changed during the storage of bottles under ambient conditions (Herath et al., 2012; Herath and Abayasekara, 2021).

However, there is a doubt that many illegal bottled water brands are coming into the market due to the increasing demand in the market. The misery behind bottled water is that the already-used bottles are refilled by some manufacturers with unhygienic water sources. Therefore, the main objective of this study was to assess the microbiological quality of bottled water available in the market in the Colombo District, Sri Lanka and to evaluate whether the quality parameters meet the permitted levels.

**MATERIALS AND METHODS**

**Sample Collection**

To determine the microbiological quality, 3 bottles from each of twenty-six (26) (currently available bottled water brands in the Colombo district – Ministry of Health, 2019) different brands of bottled water were collected randomly from local markets covering several parts (supermarkets, groceries, hotels, bus stands, and juice bars) of the Colombo District. The date of expiry and the protective polythene seal on the lid were carefully checked before the collection. All bottles were stored under ambient temperature (27±2 °C) until the time of analysis. All 26 brands were analyzed in triplicate, for each analysis. The shelf life of the bottled water is one year and all the testing was done within the shelf life.

**Determination of Total Coliforms and Fecal Coliform Bacteria**

The membrane filtration method (Obiri-Danso and Jones, 1999; Herath et al., 2012) was used to determine total and fecal coliforms. Briefly, 100 ml volumes of each sample were filtered through the membrane filtration apparatus (Pyrex, Germany) using sterilized membrane filters (Sartorius, Germany) with 0.45 µm pore size. Membrane filters were aseptically placed on pre-sterilized absorbent pads (Sartorius, Germany), saturated with 3 ml of M-Endo broth (HI-media, India) and 3 ml of M-FC broth base (HI-media, India) and were incubated for 24-48 h at 35-37 °C and 44.5 °C for the detection of total coliforms and fecal coliforms respectively. As negative and positive controls, sterilized distilled water and typical coliforms (*Escherichia coli* -ATCC 25922) were used respectively in the detection of coliform bacteria.

**Determination of Heterotrophic Plate Count (HPC) Bacteria**

Heterotrophic Plate Count (HPC) Bacteria present in bottled water samples were determined by Pour Plate Method (FAO, 1992; Herath et al., 2012). One ml of bottled water was added to a petri dish and 12 ml of nutrient agar at 50 °C was poured over it. Subsequently, the petri dish was rotated 3 times clockwise and anti-clockwise to mix well and plates were allowed to solidify at room temperature under the laminar flow. Following the drying of the plates, they were incubated at 37 °C for 48h, and subsequently, the colonies were counted. The number of bacterial colonies were reported as colony-forming units per milliliter (cfu/ml). The same analyses were carried out in triplicate for all determinations.

**Isolation and Identification of Fungal Species**

Fungi were isolated by spreading 0.1 ml aliquots of bottled water samples on Potato Dextrose Agar (PDA) plates, and the plates were incubated at room temperature for 4-5 days. Identification was done by observing colony characteristics, and reproductive morphology through light microscopic observations, and with the aid of reference
Estimation of Algal Species

Sedgwick rafter cell was used to detect algal species in the bottled water samples. 10 ml of bottled water samples were centrifuged at 4500 rpm for 5 min. Subsequently, 9 ml of supernatant water was discarded and the remaining 1 ml of water was transferred to a Sedgwick rafter cell. Algae in bottled water samples were observed in a zig-zag pattern by focusing through the medium power (10x10) of a light microscope.

Statistical Analysis

The data were statistically analyzed to investigate any significant difference among water source types in terms of the availability of HPC, Algae, Fecal Coliform, and Total Coliform by using a one-way analysis of variance at 95% confidence interval. In this analysis, the null hypothesis was considered as all means are equal whereas the alternative hypothesis was considered as at least one mean is different at the significant level of \( \alpha = 0.05 \). Furthermore, equal variances were assumed for the analysis.

RESULTS

According to the information given on the bottled water labels, natural springs, dug wells, shallow wells, and deep tube wells were widely used for bottling purposes in Sri Lanka. Water sources had been mentioned in all the brands collected. Some sources were from the central highlands and some were from the Kelani River basin. Accordingly, some sources were dug wells and it was 15%. Seventy-eight percent of the brands’ sources were tube wells and deep tube wells, 4% were from shallow wells and another 4% were from springs (Table 1).

SLS certifications were there on the label of all the bottled water and Health Ministry Registration numbers were also incorporated. Other standards such as International Organization for Standardization (ISO) and Hazard Analysis and Critical Control Point (HACCP) had been obtained by the manufacturing companies and such information was mentioned on the label. Some brands (12%) have mentioned the water purification processes such as filtration, reverse osmosis, and activated carbon filtration. The polythene seal on the lid should be there in every water bottle and it is one of the Health Ministry regulations. However, 15% out of 26 brands did not have the polythene seal on the lid. According to the Sri Lanka standards, the Health Ministry regulation in Sri Lanka TC and FC, should be absent per 100 ml of bottled drinking water. However, this study showed that 50% of the brands have exceeded the presumptive TC level (Average colony count = 21.95) and 23% of brands have exceeded the presumptive FC level (Average colony count = 1.875) of the SLSI, Health Ministry regulation and WHO (Table 2). The red colonies

<table>
<thead>
<tr>
<th>Brand</th>
<th>Location</th>
<th>Place</th>
<th>Water source</th>
<th>Location of the Water Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Kotikawatte</td>
<td>Bakery</td>
<td>Dug well</td>
<td>Horagolla</td>
</tr>
<tr>
<td>B</td>
<td>Rajagiriya</td>
<td>Supermarket</td>
<td>Deep tube well</td>
<td>Biyagama</td>
</tr>
<tr>
<td>C</td>
<td>Rajagiriya</td>
<td>Grocery</td>
<td>Tube well</td>
<td>Ranala</td>
</tr>
<tr>
<td>D</td>
<td>Himbutana</td>
<td>Sathosa</td>
<td>Dug well</td>
<td>Mandawala</td>
</tr>
<tr>
<td>E</td>
<td>Kotikawatte</td>
<td>Supermarket</td>
<td>Tube well</td>
<td>Kotagala</td>
</tr>
<tr>
<td>F</td>
<td>Kolonnawa</td>
<td>Grocery</td>
<td>Tube well</td>
<td>Padukka</td>
</tr>
<tr>
<td>G</td>
<td>Nugegoda</td>
<td>Grocery</td>
<td>Deep tube well</td>
<td>Dompe</td>
</tr>
<tr>
<td>H</td>
<td>Nugegoda</td>
<td>Supermarket</td>
<td>Spring</td>
<td>Kandy</td>
</tr>
<tr>
<td>I</td>
<td>Koswatte</td>
<td>Supermarket</td>
<td>Tube well</td>
<td>Udagama</td>
</tr>
<tr>
<td>J</td>
<td>Kirulapana (Col-5)</td>
<td>Supermarket</td>
<td>Protected shallow well</td>
<td>Rathnapura</td>
</tr>
<tr>
<td>K</td>
<td>Kirulapana (Col-5)</td>
<td>Supermarket</td>
<td>Dug well</td>
<td>Mandawala</td>
</tr>
<tr>
<td>L</td>
<td>Kirulapana (Col – 5)</td>
<td>Supermarket</td>
<td>Deep tube well</td>
<td>Horana</td>
</tr>
<tr>
<td>M</td>
<td>Maradana (Col-10)</td>
<td>Grocery</td>
<td>Deep tube well</td>
<td>Udagampola</td>
</tr>
<tr>
<td>N</td>
<td>Dematagoda (Col-9)</td>
<td>Bakery</td>
<td>Deep tube well</td>
<td>Padukka</td>
</tr>
<tr>
<td>O</td>
<td>Kurunduwatta (Col-7)</td>
<td>Grocery</td>
<td>Tube well</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>P</td>
<td>Kosgama</td>
<td>Grocery</td>
<td>Deep tube well</td>
<td>Divulapitiya</td>
</tr>
<tr>
<td>Q</td>
<td>Godagama</td>
<td>Grocery</td>
<td>Tube well</td>
<td>Bemmulla</td>
</tr>
<tr>
<td>R</td>
<td>Pettah</td>
<td>Bus Stand</td>
<td>Seal-type deep tube well</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>S</td>
<td>Pettah</td>
<td>Bus Stand</td>
<td>Tube well</td>
<td>Marawila</td>
</tr>
<tr>
<td>T</td>
<td>Pettah</td>
<td>Bus Stand</td>
<td>Deep tube well</td>
<td>Makola</td>
</tr>
<tr>
<td>U</td>
<td>Attidiya</td>
<td>Juice bar</td>
<td>Deep tube well</td>
<td>Piliyandala</td>
</tr>
<tr>
<td>V</td>
<td>Mount Lavinia</td>
<td>Supermarket</td>
<td>Tube well</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>W</td>
<td>Dehiwala</td>
<td>Supermarket</td>
<td>Tube well</td>
<td>Haliela</td>
</tr>
<tr>
<td>X</td>
<td>Dehiwala</td>
<td>Hotel</td>
<td>Tube well</td>
<td>Kurunegala</td>
</tr>
<tr>
<td>Y</td>
<td>Wellawatta (Col-6)</td>
<td>Supermarket</td>
<td>Protected tube well</td>
<td>Bandaragama</td>
</tr>
<tr>
<td>Z</td>
<td>Rathmalana</td>
<td>Grocery</td>
<td>Dug well</td>
<td>Padukka</td>
</tr>
</tbody>
</table>
with a sheen (Figure 1) and red colonies (Figure 2) were observed in M-Endo media and blue colonies (Figure 3) were observed in the M-FC media.

In the current study, 35% of samples out of the 26 brands investigated showed high heterotrophic plate counts (HPC) which exceeded the WHO quality guidelines of 50 cfu per ml (WHO, 2011) for bottled drinking water (Table 2) (Figure 4).

Eighty-five percent of the bottled water samples were contaminated with fungal species (Table 2) (Figure 5).

Among the fungal species isolated, *Aspergillus* spp., *Trichoderma* sp. and *Rhizopus* sp. were the dominant genera. In addition, *Mucor sp.* was identified.

Algae was observed in 8% (2 brands) out of 26 bottled water brands tested (Table 2). The algae species were identified as *Chlorella vulgaris* by their morphological characteristics. One of the brands which were recorded algae has mentioned that they have done many kinds of filtrations such as active carbon filtration and reverse osmosis.

**Table 2:** Numbers of each branded bottled water exceeding permitted levels of Health Ministry, SLSI, and WHO for microbiological parameters (HPC, Total and fecal coliforms and presence of fungal and algal species).

<table>
<thead>
<tr>
<th>Collected location</th>
<th>No. of brands analyzed</th>
<th>HPC</th>
<th>Presumptive Total Coliform</th>
<th>Presumptive Fecal Coliform</th>
<th>Fungi</th>
<th>Algae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>WHO, SLSI Ministry of Health</td>
<td>WHO, SLSI Ministry of Health</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colombo</td>
<td>26</td>
<td>100%</td>
<td>35%</td>
<td>50%</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>13</td>
<td>6</td>
<td>23%</td>
<td>85%</td>
</tr>
</tbody>
</table>

**Figure 1:** Detected coliform bacteria (with sheen) (a and b) in M-Endo media in 48h of incubation period at 36°C from the bottled water samples tested

**Figure 2:** Detected coliforms bacteria (without sheen) (c and d) in M-Endo media in 48h of incubation period at 36°C from the bottled water samples tested
Figure 3: Detected Fecal coliforms bacteria in M-FC media (e) in 48h of incubation period at 44.5 °C from the bottled water samples tested.

Figure 4: Detected HPC bacteria in nutrient media (f and g) in 48h of incubation period at 37 °C from the bottled water samples tested.

Figure 5: Detected fungi colonies in PDA (h and i) in 5 days of incubation period at 25 °C from the bottled water samples tested.
Table 3: One-way analysis of variance at 95% confidence interval of water source types in terms of the availability of HPC, Algae, Fecal Coliform, and Total Coliform.

<table>
<thead>
<tr>
<th>Water Source vs. HPC</th>
<th>Water Source</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>CI (95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dug Wells</td>
<td>5.93</td>
<td>8.09</td>
<td>(-14.36, 26.23)</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>Tube Wells</td>
<td>30.90</td>
<td>44.31</td>
<td>(20.75, 41.05)</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>Springs</td>
<td>0.00</td>
<td>0.00</td>
<td>(-45.379979, 45.379979)</td>
<td>0.055</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water Source vs. Algae</th>
<th>Water Source</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>CI (95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dug Wells</td>
<td>0.07</td>
<td>0.26</td>
<td>(0.0093, 0.1240)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Tube Wells</td>
<td>0.00</td>
<td>0.00</td>
<td>(-0.028690, 0.028690)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Springs</td>
<td>0.00</td>
<td>0.00</td>
<td>(-0.128304, 0.128304)</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water Source vs. TC</th>
<th>Water Source</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>CI (95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dug Wells</td>
<td>5.27</td>
<td>10.94</td>
<td>(-10.16, 20.69)</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Tube Wells</td>
<td>12.15</td>
<td>33.35</td>
<td>(4.44, 19.86)</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Springs</td>
<td>16.67</td>
<td>8.08</td>
<td>(-17.82, 51.15)</td>
<td>0.69</td>
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</table>

<table>
<thead>
<tr>
<th>Water Source vs. FC</th>
<th>Water Source</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>CI (95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dug Wells</td>
<td>0.80</td>
<td>1.47</td>
<td>(0.166, 1.434)</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Tube Wells</td>
<td>0.52</td>
<td>1.17</td>
<td>(0.200, 0.834)</td>
<td>0.72</td>
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</tr>
<tr>
<td>Springs</td>
<td>0.67</td>
<td>1.16</td>
<td>(-0.752, 2.085)</td>
<td>0.72</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Every country has its 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 it is a substance or a foreign bacterial, viral, or fungal element or a chemical substance) that may have adverse effects on human health, and each company that prepares drinking water has to follow the stipulated standards (WHO, 2011). The Ministry of Health and SLSI have stipulated the standards for drinking water in Sri Lanka.

The bottled water should be kept in places avoiding direct sunlight and in a cool dry place. 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 (WHO, 2003). In drinking water from municipal supplies, the coliform test can be used as an indicator of treatment efficiency and 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.

According to the results obtained, most of the bottled water brands exceeded the permitted level in the number of colonies presumed to be total coliforms and fecal coliforms. Similarly, research done in Sri Lanka by Herath et al. (2012) indicated that 63 % of bottled water brands tested exceeded the thresholds permitted by SLSI for presumptive total coliforms (TC) while 97 % of brands exceeded the World Health Organization (WHO) permitted limit.

Further, thirty percent of brands exceeded the limit for presumptive fecal coliforms (FC) (0 CFU per 100 mL in compliance with WHO permitted standards, SLSI, and the Sri Lanka Health Ministry regulation). Further, a study done in Jaffna Peninsula revealed that the microbiological levels of four of the eight brands analyzed were unfit for human consumption (Ragila et al., 2017). Moreover, another study done in Jaffna Peninsula by Sasikaran et al. (2012) stated that fungal and coliform bacterial contamination in 14 % and 9 % out of the 22 bottled water brands respectively, and Escherichia coli and Klebsiella spp. were found in the water brands that had fecal contamination.

Further, many studies done in different countries have reported that bottled water samples were unsuitable for human consumption due to the presence of different microorganisms. A study done in Mexico revealed that total coliforms, fecal coliforms, and Escherichia coli were found in 11 (55 %) of the water samples, 6 (30 %) of the water samples, and 2 (10 %) of the water samples, respectively. In total, 18 of the water samples (90 %) surpassed the maximum allowable limit imposed by Mexico’s standard guidelines. It is also noticed that the majority of purified bottled water samples had poor microbiological quality, with some containing Nontuberculous mycobacteria (NTM) linked to human sickness (Soria-Herrera et al., 2020). Furthermore, a study done in Pakistan stated that 33.3 % out of nine bottled water samples were within the permitted range specified by the World Health Organization (WHO) and Pakistan Standard Quality Control Authority
(PSQCA) guidelines, while 66.6 % were unsuitable for human use (Alizai et al., 2010).

According to the Bureau of Indian Standards (BIS) of drinking water, psychrophilic, coliforms, Escherichia coli, and Staphylococcal counts were deemed unfit for human consumption. However, 55 % of the samples out of twenty bottled water samples were found to be unfit for food based on the results of the total microbiological assessment (Gangil et al., 2013).

Furthermore, a study done in Kathmandu, Nepal revealed total coliform contamination was positive in 48 % of the samples, out of 100 samples. Among the coliforms, Escherichia coli was the most common species. From the 48 bottles of water, multidrug-resistant Escherichia coli, Enterobacter aerogenes, and Pseudomonas aeruginosa were isolated (Gautam, 2021). Rai et al. (2015) noticed that 75 % of the 24 bottled water samples tested had a total coliform count that was above the WHO recommendations (0 CFU/ml) and was unfit for drinking. Enterobacter aerogenes, Enteroccocus fucalis, Pseudomonas spp., Bacillus spp., and Staphylococcus aureus were also found in distinct samples (Rai et al., 2015). A study done in New Zealand examined the presence of Total Coliforms, Escherichia coli, Pseudomonas aeruginosa, Enterococci, HPC, Yeasts, and Moulds in 38 domestic and imported bottled water brands. For Total Coliforms, three brands did not meet both the Australian and New Zealand Food Standards (ANZFS) Code (2002) and the New Zealand Microbiological Reference Criteria (1995). Twenty-one brands had mold growth, while seventeen brands failed to meet HPC standards (Svagzdiene et al., 2010). Therefore, it is also noticed that the presence of coliforms and heterotrophic bacteria in bottled water is a major public health concern.

Studies have revealed that total coliforms and fecal coliforms were reported in the bottled water brands and the majority of the bottled water tested violated the manufacturer’s safety criteria (Kassenga and Gabriel, 2007; Oyedeji et al., 2010; Majumder et al., 2011 and Molfe et al., 2020). Further, according to the findings it showed that the filtration systems of the water samples similarly performed poorly and provided low drinking water quality and the existence of indicator organisms may indicate a lack of sanitation during processing (Majumder et al., 2011).

In the environment, total coliforms (TC) are present in plants, soil, etc. Consequently, the number of TCs should be higher than fecal coliforms, usually as fecal contaminants. Coliform organisms have long been acknowledged as a good microbiological indicator of drinking water quality, owing to their ease of detection and quantification in water (WHO, 2003).

The FC count rather than the TC count is a reliable measure of the pollution of water from fecal origin (Downes and Ito, 2001). The presence of FC in the samples studied is an indicator of the fecal contaminants that may lead to human diseases, such as Salmonella spp., Shigella spp., Vibrio cholerae, and other pathogens. Therefore, the positive results particularly the presumptive fecal coliform counts in some brands investigated in the current study indicate concern over the microbiological quality of bottled water in Sri Lanka.

Generally, when M-Endo, M-Endo Agar LES, M-FC, and m-ColiBlue24 are used by the National Water Supply and Drainage Board and other private laboratories, the colonies are not subjected to a confirmation test. The appearance of red colonies with a green metallic sheen on M-Endo Agar LES (MLES) and red colonies on M.coliBlue24 media are taken as positive total coliform colonies and typical blue colonies on both M-FC and mColiBlue24 are taken as fecal coliforms.

The study by Lichtigfeld and Melmed (2000) did find difficulties with background colonies on M-LES plates, some of which have been referred to as coliforms that do not create the usual colonial sheen distinguishing coliforms on this medium. In light and storage effects on the MLES, the association of sheen production with lactose fermentation and organism identification was investigated. Similarly, in the current study, some of the colonies had dark red sheen (Figure 1) and without sheen (Figure 2). These red/pink colonies appearing on the M-LES plate should not be ignored but should be subjected to confirmation of lactose fermentation on a medium such as M-LAC broth (Seidler et al., 1981). Aeromonas hydrophila also appears on M-Endo as a dark red colony, similar to the coliform which has not developed sheen. An oxidase test is recommended for the identification of Aeromonas and other positive oxidase organisms at the same time as an oxidase test for the susceptible colony to a lactose medium for the fermentation test.

Further, it is noted that pinkish-red colonies with a sheen can be observed when the plates are exposed to the sunlight for about 3 or more hours and the colony colors can be changed by keeping the medium sometime after the preparation (Lichtigfeld and Melmed, 2000).

A literature survey was conducted at the same time to examine coliforms and to interpret the findings, and it was evident that many authors had paid attention to some facts such as (Lichtigfeld and Melmed, 2000) involving background colonies in coliform membranes and coliform testing (Geldreich et al., 1978), indicators of fecal contaminations, the limits of total coliform counts (Dutka, 1973; Oger et al., 1981) and distinguishing results observed with different membranes and batches of media.

In the current study, the colonies which appeared in the fecal coliform test were dark blue (Figure 3) and the typical fecal coliform colony color in the M-FC broth is blue. However, the color of the colonies may vary with the rosolic acid and incubation temperature (Presswood et al., 1978). Studies have identified several heterotrophic bacteria as being common in bottled water; these included genera such as Achromobacter, Acinetobacter, Aeromonas, Alcaligenes, Arthrobacter, Caulobacter, Corynebacterium, Flavobacterium, and Pseudomonas (Mania et al., 1990; Bharath et al., 2003; Kassenga and Gabriel, 2007; Majumder et al., 2011; Rai et al., 2015). Some of these genera contain species that have been known as opportunistic pathogens. In the present study, 35 % of brands tested had a higher
HPC and WHO quality guidelines of 50 cfu per (WHO, 2011) for bottled drinking water. Nineteen percent of the brands were too numerous to count. The presence of a high number of heterotrophic bacteria in bottled water is probably due to the natural microbial flora of source waters. Although HPC bacteria have been considered harmless, several epidemiological studies conducted in countries such as Canada and the USA suggested the potential health risk associated with HPC bacteria present in drinking water, which comply with water quality standards (Rusin et al., 1997; Pavlov et al., 2004).

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 such as chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections, and osteomyelitis (Hedayati et al., 2007). While filamentous fungi in water do not usually pose possible problems for public health, certain fungi are isolated from mineral water. *Penicillium citrinum* and *Alternaria alternata* have some toxic potential and can pose a health risk. In regular microbiological studies on bottled mineral water, it is, therefore, advisable to count fungal propagules and lay down basal lines (Cabral and Pinto, 2002). There are several algal species in water bodies. The term ‘alga’ is used for some lower plants and many, often unrelated groups of microorganisms that can perform photosynthesis eg; *Chlamydomonas* and *Spirogyra*. One Algae species was observed in 2 brands (8 %) out of 26 bottled water brands. The alga species was identified as *Chlorella vulgaris* by their morphological characteristics. *Chlorella vulgaris* is one of the common species found in freshwater. The bottled water brand where the algae was found has mentioned that they have done many kinds of filtrations such as active carbon filtration and reverse osmosis.

According to the results of the analysis of water sources, dug wells, tube wells, and springs did not show any significant difference in terms of fecal coliform and total coliform levels in samples. Moreover, Herath (2021) stated that spring water in the knuckles mountain range, the main source of water for the bottling industry in Sri Lanka exceeded the permitted level of coliform bacteria according to the Ministry of Health regulation, Sri Lankan standards and WHO guidelines. Further, the HPC and the algae levels are significantly higher in tube wells compared to dug wells and springs at 95 % confidence interval (Table 3).

Considering the overall presence of microorganisms in bottled water, other than contaminants of source water, there is also a possibility that the plastic bottles may have been contaminated with bacteria and fungi before filling. Yoshimatsu, (1996) observed that microbial contamination can sometimes be seen in the processes of filling and capping bottles. In some bottling plants, plastic bottles are not handled or stored in sterile conditions before filling (Fujikawa et al., 1996), resulting in contamination. Besides, the methods of elimination of contaminants of source water adopted during bottling (UV and filtration) may not be sufficient for the removal of microorganisms.

**CONCLUSION**

According to the results, bottled water under some brands were unsuitable for drinking in accordance with the Health Ministry regulation in Sri Lanka (The Gazette of the Democratic Socialist Republic of Sri Lanka, 1420/4, 21/11/2005) and SLSI as they exceeded the permitted levels for one or more prescribed parameters viz; HPC, Presumptive TC, and FC. This study revealed the importance of public awareness regarding the quality and potential health risks associated with the consumption of bottled water in Sri Lanka. Therefore, the bottled water industry needs to be closely supervised by competent authorities to ensure that customers in Sri Lanka have safe bottled drinking water.

**DECLARATION OF CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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