Physicochemical and Microbiological Quality Assessment of Different Brands of Bottled Water in Maseru, Lesotho

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Abstract: Water, though vital for life, it is also the commonest route of transmission of a number of infectious diseases. Standard Methods are important for examination of the physicochemical and microbiological quality of water of any source such as bottled, tap and well water. The physicochemical parameters like hardness, alkalinity, pH were examined in comparison with WHO acceptable limits. From the microbiological point of view, water samples C, F, G and H were found to contain *Escherichia coli,* while water samples A, B, D and E were found to contain *Staphylococcus* spp., some of which are opportunistic microorganisms. This indicates poor hygienic water bottling practices, or the absence of microbiological assessment of water from the source. On the other hand, there was no significant variation observed on the physicochemical characteristics of all the different brands of bottled water including the unbottled samples and all the assessed physicochemical parameters were within the WHO standard limits. Results from the microbiological analysis indicate that potential health risks could become a concern in consumption of bottled water in Lesotho. It is therefore recommended that stricter rules should be made and implemented to regularly monitor the bottled water qualities so that the labels of bottled water must include not only the pristine glaciers and Himalayan springs but also the relative concentrations of water quality parameters. Moreover, all the bottled water companies should fulfill the basic water quality standards given by the WHO. Awareness should also be created to the public for either using disinfectants or boiling water before use rather than rely on the belief of purity.

Keywords: Physicochemical, Microbiological, Quality assessment, Bottled water and Lesotho.

1. Introduction

Water is very vital for the existence of all living organisms. This valued resource is increasingly threatened as human populations grow and demand more water of high quality for drinking and for other domestic and economic activities [1]. Though 70% of the earth is covered by water, fresh water accounts 3% of the total water on earth [2]. Majority of freshwater are locked as glacier and polar ice which is difficult to utilize and importing them is costly. As fresh water resources are further stretched to meet the demands of industry, agriculture and an ever expanding population, the shortage of safe and accessible drinking water is estimated to become the major challenge in many parts of the world. There are two major problems with the fresh water of the earth: Fresh water shortage and water quality. Drinking water is derived from two basic sources: surface waters, such as rivers and reservoirs, and groundwater. All water contains contaminants, particularly inorganic contaminants that arise from the geological strata through which the water flows and, to a varying extent, anthropogenic pollution by both microorganisms and chemicals. In general, groundwater is less vulnerable to pollution than surface waters.

Although water is vital for life, it also serves as the commonest route of transmission of a number of infectious diseases. Thus, water quality must be ensured before drinking and the water we drink must be safe. Water quality is defined as the ability of a water body to support all appropriate beneficial uses such as drinking, irrigation or any other purpose, and the determination is typically made relative to the purpose of the water. The quality of water is reflected by various physical, chemical and biological conditions which in turn are influenced by natural and anthropogenic sources. Safe drinking water is defined as water with microbial, chemical and physical characteristics that meet WHO guidelines of national standards on drinking water quality [3]. Water quality parameters like alkalinity, hardness, Dissolved Oxygen (DO), chloride, Total Dissolved Solid (TDS), etc., add to the aesthetic value of water, while parameters like

Figure 1. Five different brands of bottled water.

Figure 2. Well water source, Roma.

ammonia, lead, arsenic, nitrate etc may cause adverse health effects. Water having high or low pH, greater extent of turbidity etc., is objectionable to use. Appropriate amount of chloride content and hardness are desirable but higher content of the same makes the water unaesthetic. Similarly higher content of phosphate, nitrate, ammonia, iron, are undesirable. Some other chemical constituents like arsenic, lead etc. may be toxic.

From microbiological point of view, drinking water should be free from any kinds of pathogens as well as opportunistic microflora. Although there are a number of microorganisms present in water that may pose health threat like *Salmonella* spp., *Shigella* spp., Coliforms, *Mycobacterium* spp. etc. Coliforms are used to assess water quality. Coliforms are gram negative, rod shaped bacteria capable of growth in presence of bile salts and able to ferment lactose at 35-37º C with the production of acid, gas and aldehyde within 24-48 hours. They are oxidase negative and non-spore forming. Coliform organisms (*E. coli*) have long been recognized as a suitable microbial indicator of drinking water quality largely, and thus, their presence in water samples indicates the presence of fecal matter and the possible presence of pathogenic organisms of human origin [4].

The micro-organisms in water are capable of causing various diseases like typhoid (caused by *Salmonella enterica*serovarTyphi), cholera (caused by *Vibrio cholerae*), diarrhea (caused by *E. coli* O157:H7), dysentery (caused by *Shigella dysenteriae*), hepatitis (caused by Hepatitis viruses A, B, C, D, E), etc. According to WHO, unsafe water supply is a major problem and fecal contamination of water sources and treated water is a persistent problem worldwide [3]. Globally, 1.1 billion people rely on unsafe drinking water sources from lakes, rivers and open wells. The WHO has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, pollution or unavailability of water. Hence it is necessary to purify and disinfect water before it is available for drinking. Many researches and studies have revealed that tap water does not ensure the quality of water. According to the National Water Quality Association, 56% of all people are worried about the quality of municipally treated tap water. With the rising concern on public health, people choose bottled water over tap water.

Bottled water is a term referring to water that is presumed to be processed, packaged and sold in containers or simply bottles. According to the International Bottled Water Association, “Bottled water is a great beverage choice for hydration and refreshment because of its consistent safety, quality, good taste and convenience”. Bottled water can be categorized into Artesian well water, distilled water, mineral water, purified water, sparkling water, well water etc. according to their source and state of purification. From 1988 to 2002, the sales of bottled water globally have more than quadrupled to over 131 million cubic meters annually. One of the main reasons of this is the compromised water quality provided by municipality. Another reason may be the public perception that the bottled water is essentially of high quality.

The public perception and probably the reality is that bottled water is regularly of high quality. This belief is encouraged by publicly reported problem of municipal tap water as well as the public perception of purity driven by advertisements and packaging labels featuring pristine glaciers and crystal clear mountain springs. However, many studies have shown that these beliefs need not always be true. A four-year study conducted by the National Resources Defense Council (NRDC) revealed that about one-third of the samples contained significant contamination, including synthetic chemicals, bacteria and arsenic, in at least one sample, out of more than 1000 samples of 103 bottled water brands tested. The objective of this study was therefore, to assess physicochemical and microbiological quality of different brands of bottled water in Maseru, Lesotho.

2. Materials and Methods

Water sample sources:

Five available brands of bottled water were used in the study for physicochemical and microbiological assessment. From each of these brands, twelve bottles of water (500ml) were collected (figure 1) from different supermarkets in Roma and Maseru. Tap and well water (figure 2) samples were also assessed for the physicochemical and microbiological quality for comparison. The samples then taken to Biotechnology laboratory, Department of Biology, National University of Lesotho and assessed for quality (physicochemical and microbiological assessment). The samples were kept at 4º C in the fridge when not used.

Water sample analysis:

Physicochemical parameters:

- **pH**

pH was measured by automatic digital pH meter. The pH meter was first calibrated with a standard buffer solution. The glass electrode was washed with distilled water. Then glass electrode was dipped in the beaker containing water sample until the reading was stabilized at a certain point. Then pH reading was noted down.

- **Electrical Conductivity**

The instrument used was digital conductivity meter. The conductivity electrode was dipped in the beaker containing water sample until the reading was stabilized at a certain point. Then reading was noted.

- **Chloride**

Chloride was measured by titration method [5]. About 2 mL of Potassium chromate was added to the sample solution of about 50mL. It was titrated against 0.02N silver nitrate until a persistent brick red color appeared which was the end point of the titration. A blank by placing 50 mL of chloride free distilled sample water

Figure 4. Cream white colonies on MSA.

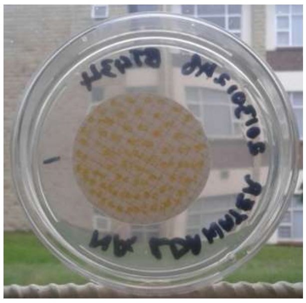

Figure 3. Bacterial colonies after filtration of one brand of bottled water.

was also conducted.

Calculation

Chloride (mg/L) = $\frac{(a-b) \times N \times 35.5 \times 1000}{V}$

Where, a = Volume of titrant (silver nitrate) for sample

b= Volume of titrant (silver nitrate) for blank

V = Volume of the sample in mL

N = normality of silver nitrate

- **Total hardness**

Total hardness was determined by EDTA method [5]. This was done by titrating 100mL of the water sample in a conical flask and adding 1mL of buffer solution with Erichrome Black-T indicator against standard EDTA (Ethylene diamine tetra acetic acid). The solution changed from wine blue at the end point was recorded as positive for metallic cation. Total hardness might be caused by the sum of all metallic cations other than alkali metals and expressed as equivalent calcium carbonate concentration.

Total hardness (as CaCO3), (mg/L) = $\frac{\text{mL of EDTA used} \times 100}{\text{mL of sample}}$

- **Free CO_2**

Free CO_2 in water was determined by using titration method [5]. For this, about 100 mL of sample was taken and 2 drops of phenolphthalein indicator were added. Then the solution was titrated against 0.05N of NaOH from the burette until pink color appeared. The following formula was used to calculate free CO_2 concentration (mg/L).

Free CO_2 (mg/L) = $\frac{A \times \text{Normality of NaOH} \times 44 \times 1000}{\text{Volumeofsampleinml}}$

Where, A = Volume of NaOH used in mL.

- **Total Alkalinity**

Total alkalinity of water was determined by titrimetric method [5]. About 100mL sample with 2-3 drops of methyl orange was titrated against standard, 0.02N H2SO4. At the end point, the colour changed from yellow changed to pink and this was recorded as positive for alkalinity.

Total Alkalinity (mg/L) = $\frac{a \times N \times 1000 \times 50}{\text{Volume of sample in mL}}$

where, a= Volume of standard H2SO4 consumed in titration

N= Normality of H_2SO_4 used

- **Nitrate-N**

Nitrate content in the water sample was determined by Phenol disulphonic acid method. In this method, about 50 mL of filtrate sample was taken in a porcelain basin and evaporated to dryness. It was cooled and residue was dissolved in 2 mL of phenol disulphonic acid and diluted to 50 mL. About 6 mL of liquor ammonia was then added to develop yellow colour. Then the reading was taken at 410 nm on spectrophotometer. Same procedure was repeated for the standard solution of different concentration and for distilled water. Then the concentration of Nitrate-N was determined from the standard curve obtained by plotting standard value against absorbance.

Microbiological analysis:

Most Probable Number (MPN): The most probable number/ multiple tube fermentation test was used to detect and quantify Coliform bacteria [5]. This test was performed sequentially in three stages: presumptive, completed and confirmed tests.

Presumptive Test: The presumptive Coliform test was done to detect and estimate the Coliform population in the water samples. Volumes of 10ml, 1ml and 0.1ml of each water sample were inoculated into 10ml of lactose broth, and the test tubes were incubated at 37ºC for 48 hours. The tubes were then observed after 24 and 48 hours. The production of any gas within any 24 hours in any of the tubes was recorded as a positive presumptive test while the production of gas during the following 24 hours was recorded as a doubtful test. The absence of gas production after 48 hours of incubation showed a negative test, hence indicating the absence of Coliforms. Coliforms in the test tubes were then expressed as the most probable number (MPN) of Coliforms, which is the count of the number of lactose fermentation tubes showing production of gas following the incubation period. The MPN value was gross referred by matching the results obtained with those in the statistical table provided.

Confirmed Test: The confirmed test was done to prove the presence of Coliforms in the positive and/ or doubtful presumptive tests. Samples from the positive and doubtful presumptive lactose broth tubes were spread plated onto a selective differential medium for Coliforms- Eosine Methylene Blue (EMB) Agar in a discrete manner. The plates were incubated at 37ºC for 24-48 hours and then evaluated for the appearance of typical Coliform colonies with dark centers and metallic sheen.

Completed Test: The completed test was done to confirm the presence of Coliforms in the water samples. Lactose-positive colonies from EMB agar

Figure 5. Light pink colonies on EMB.

plates were isolated, inoculated into a lactose broth tubes and streaked on nutrient agar slants so that biochemical tests could be performed. For positive completed test, the production of gas and acid in the inoculated lactose broth and the presence of Gram-negative rod-shaped bacteria confirmed the presence of Coliforms in the water samples.

Membrane Filtration (MF) Technique: Appropriate nutrient or culture media were selected (specifically nutrient agar). The membrane filters removed from the sterile package using the forceps and the membrane filters were then placed into the funnel assembly and the samples were poured into the funnel. The membrane filters were removed from the funnel allowing the liquid to draw completely through the filter and then placed into a prepared nutrient agar in the Petri dishes. The Petri dishes were then incubated at appropriate temperatures (37ºC) for appropriate time (24-48 h) and the colonies were then counted. The colonies from different brands of water were purified and then grown on different selective media (Bacillus Cereus Agar, Brilliant Green Agar, Violet Red Bile Glucose Agar, Eosine Methylene Blue Agar, MacConkey Agar, Mannitol Salt Agar and Salmonella Shigella Agar) for identification.

Biochemical tests: Biochemical tests that include IMViC, catalase, and Gram-staining were performed (figures 3, 4 and 5) to confirm the presence of coliforms and other heterotrophic bacteria in the water samples and also to identify the isolates to genus level.

Data analysis: BM SPSS Statistics version 20 was used to analyse the mean variation between samples for the tested parameters.

3. Results and Discussion

Physicochemical parameters:

On average, water sample H had the highest pH value, then followed by water samples C, G, F, E, B, A and D (Table 1). Water sample H also had the highest value of electrical conductivity, and then followed by C, D, B, A, G, then F and lastly by E (Table 1). Chloride content, Total Hardness, Free CO_2 and Total Alkalinity were found to be in the range of 0.012-0.120 mg/L, 1.6-30.8 mg/L, 2.2-30.8 mg/L, 3.3-7.5 mg/L and 0.04-0.07 mg/L respectively (Table 1). The water brands A and C showed high electrical conductivity and total alkalinity compared to other water samples (Table 1).

Unbottled water from different sources and all the values are well below the permissible limits of WHO [6]. However, the assessed physicochemical parameters of all the five different brands of bottled water did not correlate with those on the labeling and this shows that most companies just bottle water from

Table 1: Physicochemical parameters of different brands of bottled water and unbottled water from different sources in Maseru, Lesotho.

Water sample	pH	EC (µS/cm)	Chloride (mg/L)	Total Hardness (mg/L)	Free CO2 (mg/L)	Total Alkalinity (mg/L)	Nitrate (mg/L)
A	7.73±0.02	7.64±0.15	0.08±0.04	2.40±0.00	2.93±1.27	3.63±0.12	0.06±0.01
B	7.59±0.03	1.37±0.15	0.05±0.02	1.87±0.23	4.40±0.00	3.37±0.06	0.05±0.01
C	8.09±0.05	89.20±0.69	0.02±0.02	4.93±1.01	12.47±1.27	7.33±0.15	0.06±0.01
D	7.42±0.10	30.83±0.67	0.02±0.02	6.67±1.16	4.40±0.00	3.27±0.06	0.04±0.01
E	7.87±0.02	0.39±0.01	0.04±0.01	29.87±1.01	27.87±2.54	3.83±0.06	0.04±0.00
F	7.90±0.01	0.47±0.00	0.04±0.00	29.60±0.69	29.33±2.54	3.90±0.00	0.05±0.01
G	7.91±0.01	0.48±0.00	0.04±0.01	32.27±1.01	32.67±2.54	3.90±0.10	0.05±0.01
H	8.31±0.02	155.83±1.17	0.02±0.02	4.93±1.01	4.93±1.27	7.40±0.10	0.07±0.00

Key: EC = Electrical Conductivity

Table 2: MPN values of different water samples.

	Volume used (mL)			
Water sample	10	1	0.1	Total Count (MPN/100mL)
F	3	3	3	>11
G	3	3	3	>11
H	3	3	3	>11

Table 3: Number of colonies per brand of bottled water and the colour of those

	Number of colonies (CFU/mL)				
Water sample	Trial 1	Trial 2	Trial 3	Average	Colour of colonies
A	141	77	52	90	White
B	83	45	59	62	White
C	TNTC	TNTC	TNTC	TNTC	White
D	67	57	57	62	White
E	60	59	59	59	White

Table 4: Microbial growth and characterization on different selective media.

		Presence/ Absence of microorganisms		
Water sample	Selective media	Trial 1	Trial 2	Trial 3
A	MacConkey Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies
	Mannitol Salt Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies
B	MacConkey Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies
	Mannitol Salt Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies
C	Eosine Methylene Blue Agar	+++ Light pink colonies	+++ Light pink colonies	+++ Light pink colonies
	MacConkey Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies
D	MacConkey Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies
	Mannitol Salt Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies
E	MacConkey Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies
	Mannitol Salt Agar	+++ Cream white colonies	+++ Cream white colonies	+++ Cream white colonies

the source with little or no water quality assessment at all. The results showed no significant variation from different brands of bottled water compared to unbottled water (Table 1).

Microbiological Analysis:

Most Probable Number: The MPN Index and 95% Confidence Limits for various combinations of positive tubes in a 3 tube dilution series using inoculums quantities of 10, 1 and 0.1ml (Table 3.0), it was found that the MPN value of water samples F, G and H was >11 MPN/100ml (Table 2) and this was above the WHO recommendation (6).

Membrane Filtration (MF) Technique: On average, water sample C was found to contain the highest number of microorganisms, then followed by water sample A and then followed by both B and D (Table 3). On the other hand, water sample E was found to contain the least number of microorganisms, and it was also found that these five brands of bottled water contained white colonies (Table 3).

It was found that the microorganisms present in water samples A, B, D and E could only show growth on MacConkey Agar and Mannitol Salt Agar and no growth on Bacillus Cereus Agar, Brilliant Green Agar, Violet Red Bile Glucose Agar, Eosine Methylene Blue Agar and Salmonella Shigella Agar. On the other hand, microorganisms from water sample C could only show growth on Eosine Methylene Blue Agar, MacConkey Agar and no growth on Bacillus Cereus Agar, Brilliant Green Agar, Violet Red Bile Glucose Agar, Mannitol Salt Agar and Salmonella Shigella Agar (Table 4).

Characterization of isolates:

Water samples A, B, D and E were found to contain *Staphylococcus* spp., some of which are opportunistic microorganisms (for example, *S. aureus*) while water samples C, F, G and H were found to contain *Escherichia coli* (Table 5). *E. coli* O157:H7 is one of hundreds strains of the bacterium *E. coli*. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and thus can cause severe illness [4]. Infection often causes severe bloody diarrhea and abdominal cramps. In some people, particularly children under 5 years of age and the elderly, the infection can cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail [4].

4. Conclusions and Recommendations

Hence from the above results, it can be concluded that bottled water, although thought to be safe, cannot be relied upon for its safety. All the physicochemical parameters like hardness, alkalinity, pH were within WHO acceptable limits. Nitrates were found in small quantities but within the limits set by WHO. Thus from the physicochemical aspect, the quality of water is good. However, the results on the physicochemical aspect of water quality showed no significant variation from different brands of bottled water compared to unbottled water and the physicochemical parameters of the five different brands of bottled water did not correlate with those on the labeling.

From the microbiological point of view, all the water samples were found to have a potential to cause some illnesses to human beings because water samples A, B, D and E were found to contain *Escherichia coli,* while water samples C, F, G and H were found to contain *Staphylococcus* spp., some of which are opportunistic microorganism. As per the WHO result says, “While much tap water is indeed risky, having compared the available data, we conclude that there is no assurance that bottled water is any safer.” Similar is the conclusion of this study, that there is no assurance that since water comes out of a bottle does not mean it is free from contamination.

It is therefore recommended that stricter rules should be made and implemented to regularly monitor the bottled water qualities so that the labels of bottled water must include not only the pristine glaciers and Himalayan springs but also the relative concentrations of water quality parameters. Moreover, all the bottled water companies should fulfill the basic water quality standards given by the WHO. Awareness should also be created to public for either using disinfectants or boiling water before use rather than rely on the belief of purity.

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