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COMPARATIVE STUDY OF MICROBIOLOGICAL, CHEMICAL AND PHYSICAL PROPERTIES REGARDING THE QUALITY OF BOTTLED WATER SAMPLES COLLECTED FROM DIFFERENT COUNTRIES

Public Health

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ABSTRACT

Introduction: People rely on the quality of the bottled drinking water, expecting it to be free of microbial contamination and health hazards. Objectives of a comparative study of microbiology, chemistry, and physics on the quality of bottled water samples from different countries.
Methods: All bottled water samples sold and collected. Electrical conductivity, total dissolved solid, pH, calcium, nitrate, total aerobic and anaerobic count, coliform bacterial count and fecal contamination were checked.
Results: These are 34 brands of bottled drinking water was used. The sample most type of bottled water sample shows an increase in mineral results was 33, 45 and 46, NO3, Fe, NO2, and F was within normal range in all samples. The biological and bacteriological test was almost normal in all samples, heavy metals show an increase in most of the study sample.
Conclusions: The bottled drinking water available for sale do not meet the standards stipulated, it is necessary to check the sample from each brand.

KEYWORDS

bottled water, microbiological properties, chemical properties and chemical properties.

INTRODUCTION

The key to increase human productivity and long life is good quality water **(E.T. Urbansky et al, 2002)**. The provision of good quality household drinking water is often regarded as an important means of improving health **(S. Moyo et al, 2004)**.

According to World Health Organization **(WHO, 2002)**, there were estimated 4 billion cases of diarrhea and 2.2 million deaths annually. The consumption of unsafe water has been implicated as one of the major causes of this disease. Most gradual deterioration of water quality was resulted by the increase in human populations and urbanization **(K.C. Ho et al, 2001)**. As water pollution is getting serious, houses especially in the urban area started to equip with a water filter system. People are concern with the presence of pollutants such as heavy metals and toxic chemical in their daily drinking water. Filtered water is the main source of safe and reliable drinking water. However there is still a debate on the efficiency of filtration system to comply with the regulations as water that physically looks colorless, odorless and even tasteless is not sufficient to determine that the water is safe for consumption. In fact, the drinking water should be examined on microbiological and physicochemical quality. The **WHO in its 2002** report, recommended that increased emphasis be placed on home water treatment and storage, and that more research should be conducted to assess the health benefits of such interventions. Contaminants can be in the form of microorganism that barely visible in unaided eyes. A number of authors have reported a statistically significant deterioration in the microbiological quality of water between the source and point of use in the home **(C. Simango et al, 2002; B. Genthe et al, 1997; P. Welch et al, 2000)**.

According to WHO **(WHO, 1996)**, the physical parameters that are likely to give rise to complaints from consumers are color, taste, odor, and turbidity while low pH causes corrosion and high pH results in taste complaints. Therefore the objective of this study was to determine the microbiological and physicochemical quality of water samples collected before and after the filtration treatment given.

The quality of drinking-water may be controlled through a combination of protection of water sources, control of treatment processes and management of the distribution and handling of the water.

Recently, the consumption of bottled mineral waters has increased worldwide, because people living in developed countries have no suitable water supply at their homes **(A. Azlan et al, 2012)**. Natural mineral waters are defined as a microbiological healthy water originating in a groundwater tables or deposits and emerging from a spring tapped at one or more natural or bore exits with an individual and constant chemical composition **(D. Bertoldi et al, 2011)**. Casanovas-Massana and Blanch described that natural mineral waters are complex environments containing a large variety of autochthonous microbiota **(A. Casanovas-Massana et al, 2012)**. Mineral water is characterized by its purity at source, its content in minerals, trace elements, and other constituents **(L. Petraccia, et al, 2006)**. Falcone-Dias et al. reported that mineral waters have been marketed as ideal for infant formula preparation and nursery drinking water, particularly for the immunosuppressed people **(M.F. Falcone-Dias et al, 2012)**. In assessing the quality of drinking waters, consumers rely principally upon their senses with bottled waters being perceived as pure, safe, and of good taste; thus, their consumption is increasing despite the excessively high prices compared to tap water **(G.R. Kassenga, 2007)**. Bottled mineral waters are relatively large proportion of bottled water (between 40 and 60% globally) **(M.F. Doria, 2006)**. Bottled drinking water has been occasionally related to diarrhea conditions known as traveller's disease. Although the microbial quality levels in processed water are often initially low, bottled mineral water is not a sterile product and can contain naturally occurring bacteria as well as those introduced during manufacturing or consumer handing **(G.R. Kassenga, 2007; A. Zeenat et al, 2009)**.

Mineralized water can also offer a significant amount of required nutrient such as calcium and magnesium **(B.H. Jin et al, 2010)**. Other components such as carcinogenic compounds, insecticides as DDT, and heavy metals **(M. Cemek et al, 2007)**, generally, are present in mineralized water in trace concentrations, whose effects on human health are inadequately understood or unknown **(B.H. Jin et al, 2010)**. Contamination of bottled water with nitrate and nitrite affects the health of infants more than adults, as they are more susceptible to serious health conditions such as Methemoglobinemia, due to the difference in their body size and chemical conditions of the body **(M.Y.Z. Abouleish, 2012)**. Cidu et al. testified an evaluations on the quality of drinking waters. They found that concentrations of Cl^-, F^-, and As^- were higher than water quality guidelines **(R. Cidu et al, 2011)**. Chloride, sodium, and potassium amount introduced through water is actually very small, and magnesium and calcium content varies remarkably depending on the kind of water **(L. Petraccia, et al, 2006)**.

AIM OF THE WORK:

This study aim to comparative study of microbiology, chemistry and physics on the quality of bottled water samples from different countries.

MATERIAL AND METHODS

The samples were collected using the sampling and storage procedures according to Benjamin and Brown (A.P. Benjamin et al, 2003) [21]. All samples were collected in July 2017 and samples were kept in 1000 ml sterile autoclavable plastics (polypropylene) bottles, labelled with different codes and immediately stored at 4°C when transported back to the laboratory from sample collecting site.

Gram Staining

The chemicals and media used for this study were from OXOID and MERCK. The microbiological analyses performed on the samples were total viable count using the method from **Roberts et al, 1995**, bacteria were identified using Gram staining. A loopful of bacteria grown on R2A agar was placed on a clean slide and air dry by slight heating. The slide was colored using crystal violet for 1 min and rinsed off with water. A few drops of alcohol were used to discolor the crystal violet and immediately rinsed off the alcohol with flowing water. The remaining stain was then washed by safranin and the slide was examined under a microscope.

Coliforms, Faecal coliforms and Escherichia coli (E. coli) (Preliminary and Confirmation test)

Enumeration of viable cell was done according to **Roberts et al, 1995**. A serial decimal dilution was prepared using Maximum Recovery Diluents (MRD). The original water samples were diluted (10^{-1} -10^{-4}) and pipetted on R2A using spread plate method and incubated at 20-22°C for three days. Gram staining suggested by Roberts and his colleagues **(Roberts et al, 1995)**, were used to identify bacteria present on the R2A agar. One hundred mL samples were filtered through 0.45µm pore size cellulose nitrate membrane filters (Sartorius). Membrane filters were subsequently transferred face upwards onto Endo Agar using a sterile forceps. The agar was incubated at 35°C for 18-24 hours. A confirmation test on the presence of coliforms, faecal coliforms and E. coli, were performed by inoculating culture into two Brilliant Green 2% Bile Broth (BGBB) test tubes with each having a Durham test tube and test tube containing 1% tryptone water (TW). One BGBB test tubes were incubated at 37°C for 48 hours while the other BGBB and TW tubes were incubated at 44°C for 24 hours. About 0.2-0.3 mL Kovac's Reagent were added to TW to detect indole formation, (a red color layer on surface). Gas released in Durham test tubes indicated presence of coliforms, faecal coliforms and E.coli.

Streptococcus faecalis and Pseudomonas aeruginosa

A detection test on the presence of Streptococcus faecalis was done. One hundred mL samples were filtered through cellulose nitrate membrane filters. Membrane filters were subsequently transferred face upwards onto KF Agar using a sterile forceps. The agar was then incubated at 35°C for 48 hours. Pink color colonies with a diameter of 0.5-2.0mm showed the presence of S. faecalis. A further confirmation test was done by inoculating the colony onto Kanamycin Esculin Azide Agar and incubated at 35°C for 72 hours. A catalyst test was used to identify *S. faecalis*. The detection of *P. aeruginosa* was done by placing filtered cellulose nitrate membrane filters onto basic Pseudomonas Agar. The media was incubated at 42°C for 40-44 hours. Later the agar was checked under ultra violet light to detect pigment thought to be *P. aeruginosa*.

Determination of Water Quality Parameters

The samples were also evaluated on the physicochemical aspects to determine turbidity, total suspended solid (TSS) and pH.

Turbidity:

Turbidity in Nephelometric Turbidity Units (NTU), was measured using a HACH ratio turbidometer (Model 2100 AN, Co., USA). A total of 15mL sample was placed in a special container and inserted into the turbidometer for reading.

pH:

WTW pH 420 meters were used.

TSS:

The Total Suspended Solid (TSS) was according to **Mazlin et al, 2003**. A filter paper was weighed and later wet with sterile distilled water. The sample was stirred homogenously to ensure no precipitation. The sample was measured accurately and poured through the filter paper. The filtering was done using a suction pump. Next the filter paper was transfer into the oven for drying at 103°C for 24 hours. The filter paper was left to cool before being weighed. The TSS was counted according to the formula below:

$$\text{mg of total suspended particles/ L} = \frac{(A-B)x1000}{ml\ sample}$$

Where, A = filter paper weight + residue after filtering and drying (mg), and B = filter paper weight (mg).

RESULTS AND DISCUSSION:

This study was carried out on 34 analyzed brands of bottled mineral water samples in this research were the most consumable and very high sales brands between other bottled mineral waters. They were obtained from shops in the city in the period from Jan. 2017 till June 2017. The bottled mineral water samples were opened in the laboratory and analyzed.

Mineral Results

Table 1 shows ranges, mean, and standard deviations of results comparing to water quality guidelines regarding mineral results, it was found that, all sample within normal range of NO_3, Fe, NO_2 and F. while only one sample was out of range in CL, Ca, SO_4, Cu and K, two sample out of range of Mg, three sample was out of range regarding TDS and T.H., the height number of sample out of range was found in Na. Sample Number 45 show out of range followed by sample number 33 and 46. Vandevijvere et al. reported that the mineral levels were different between label and water contents of analyzed bottled mineral waters in Belgium **(S. Vandevijvere et al, 2009)**.

Azlan et al. presented that concentrations of manganese and fluoride in mineral waters were slightly lower than the international standard recommended limits in Malaysia **(A. Azlan et al, 2012)**. However in our study, the fluoride results were higher than water quality guidelines. Cidu et al., in 2011, stated that in 20% of the bottled water samples, one or more components have been found at concentrations exceeding the Italian regulations (Cl−, SO42−, NO3 −, F−, and As) **(R. Cidu et al, 2011)**. Also, in the present study, concentrations of these ions were exceeded the guidelines which were in opposite of this study results.

Cemek et al. indicated that nitrate and nitrite levels in the mineral waters were not found in concentrations considered to be hazardous in terms of public health **(M. Cemek et al, 2007)**. Also, Bertoldi et al., based on survey of the chemical composition of 571 European bottled mineral waters, reported that according to European legislation, 9% of samples had boron, nitrate, or nitrite levels above the legal limit existing in individual European **countries (D. Bertoldi et al, 2011)**. Abouleish described that all bottled water samples demonstrated nitrate, nitrite, and other anions levels below the permissible levels accepted by U.S-EPA, U.S-FDA/CFR, and other international organizations, levels exceeding the European commission and drinking water directive (EC/DWD) permissible levels **(M.Y.Z. Abouleish, 2012)**, and in our study the mean of all ions except potassium and magnesium had values less than codex guideline values **(C. Standard, 2008)**. Chiarenzelli and Pominville showed that except for one sample of mineral water, all bottled waters tested meet United States Environmental Protection Agency (US-EPA) primary standards for drinking water supplies **(J. Chiarenzelli et al, 2008)**.

Biological results:

Conductivity

Electrical conductivity is a measure of water capability to transmit electric current and also it is a tool to assess the purity of water. Electrical conductivity found in the range up to 800 ms/cm **(N. Shrivastava et al, 2013)**. Only two samples was out of range (Number 33 and 45). One of the reason of salinity is the high concentration of captions such as sodium, calcium and magnesium whereas chloride, phosphate and nitrate as anions **(P.A. Raja, 2008; R. Chauhan et al, 2012)**.

pH:

pH is affected not only by the reaction of carbon dioxide but also by organic and inorganic solutes present in water (Table 2). Any alteration in water pH is accompanied by the change in other physic-chemical parameters **(N. Shrivastava et al, 2013)**. pH maintenance (buffering capacity) is one of the most important attributes of any aquatic system since all the biochemical activities depend on pH of the surrounding water. It was concluded that the pH of water were slightly alkaline (7.5 to 8.5) and were within the maximum limit set for domestic use as per APHA. High value of pH may results due to waste discharge, microbial decomposition of organic matter in the water body **(P.A. Raja, 2008; R. Chauhan et al, 2012)**. The high pH in this case may be attributed to sewage discharge by surrounding human population. Only one sample (Number 33) shows a low pH.

Color, odor and taste:
None of the bottled sample had any color, odor or taste.

Bacterial contamination
According to the **Sri Lankan Standard (894, 2003)**, acceptable aerobic count is from 1×102 to 1×104 cfu /mL. The aerobic bacterial counts of K, S, L and M; B, O, H and N; R, V, P, A and I and L, C, Q, D, U and T brands were closely related to each other. In our study none of the bottled drinking water brands contained anaerobic bacterial growth.

Fungal contamination
Of the 34 bottled drinking water tested, no one contained fungal contamination. **Sasikaran S. et al 2012**, found of 22 bottled examined, some bottled had Mucor spp contamination and had Aspergillus spp contamination.

Heavy metals:
Heavy metals enter the environment via natural and anthropogenic means. Such sources include industrial discharges, mining, erosion, sewage discharge water, waste effluents etc. the main route of exposure for most people is through food and water. Consistent exposure to heavy metals at low levels can cause great adverse effects **(WHO 2008; ATSDR 2008; V. Mudgal et al, 2010)**. Heavy metals have toxic effects on humans, animals, fisheries and plants. They are persistent in the environment and they bioaccumulate in plants and human beings **(WHO, 2006; B. Mridul et al, 2013; M. Castro-Gonzalez et al, 2008)**.

From the analysis carried out on the water samples, it was discovered that sliver, bismuth, cadmium, mercury, lead, Tin, tantalum and thallium levels in the sample were within WHO limits.

Beryllium was increase in only one sample (2.9%) in sample No. 33, Cesium and HCO3 increase in two sample only (5.9%), Sample number 33 show increasing in both cesium and HCO3 and sample number 62 increased in cesium only and sample number 45 was increased in HCO3.

David T. et al 2012, carried study on investigation of heavy metals in drinking water, Mg), Calcium (Ca), Manganese (Mn), Iron (Fe), Zinc (Zn), Copper (Cu), Lead (Pb), Chromium (Cr), Cadmium (Cd) and Nickel (Ni), and from the result and analysis, only Mg, Ca, Fe, Mn and Zn, where observed. the concentration of Mg is between 0.06 mg/L and 0.73mg/L. when compared with WHO (2005) standand of 50mg/L, the concentration is low and not toxic. The concentration of Calcium (Ca) from table 1 and figure 2 indicate a relatively low concentration in the study area. Ca falls within the range of 0.1mg/L and 2.86mg/L as against toxicity limit of WHO (2005) of 200mg/L. Iron (Fe) concentration in water sample was between 0.15mg/L and 0.28mg/L. Though not above the WHO (2005) limit, the concentration of Fe in B2 at 0.28 and P1, P2 at 0.28 appears high, the concentration is still below toxicity limit.

Zinc (Zn) levels vary from a minimum of 0.23mg/L to maximum of 0.89mg/L. The concentration is still within the WHO (2005) limit, and is not toxic for both sachets and bottle waters. The concentrations of Manganese (Mn) in the samples varied from a minimum of 0.01mg/L to a maximum of 0.04mg/L. Concentration of Mn is low in Bottled water between 0.01mg/L and 0.03mg/L. From the study, it is observed that the following heavy metals were not detected. They include; Copper (Cu), Cadmium (Cd), Lead (Pb), Chromium (Cr), and Nickel (Ni). This absence does not pose any health implication to the resident of the study area. In out study Aluminum, arsenic, boron, barium, bromine, chromium, cupper, iodine, lithium, manganes, nikel, ubidium, antimony selenium, strontium, uranium and zinc show a significant increase in at least 20.0% of the sample.

CONCLUSION:
It is necessary that the sachet water business be critically reviewed by special laboratory to ensure that producers comply within standards. It is importance that regulatory authorities intensify their inspection activities in this ever growing area of sachet water production as they should ensure that producers adhere strictly to the stipulated standards to avoid public health hazards.

Table (1): Ranges, mean, and standard deviations of results compared to water quality guidelines in mineral results.

Parameters	Normal range	Range of sample	Mean±SD	No. of samples out of range	Percent	Name of sample
TDS	228	856-2	155.6±129	3	8.8	33, 45, 46
CL	250 mg/l	278-4	47.02±30	1	2.9	33
T.H	400	800-0	164.5±145	3	8.8	33, 45, 46
Ca	100-200	256-0	46.87±35	1	2.9	45
Mg	30-50	54.9-0	15.77±15	2	5.9	31,33
No_3	25-50	22.6-5	3.508±8.6	0	0.0	-
Fe	1 mg/L	0.07-0.01	0.018±0	0	0.0	-
So_4	25-250	675-2	113.7±35	1	2.9	45
No_2	5 mg/L	0.02-0.002	0.004±0	0	0.0	-
Cu	2 mg/L	2.17-0	0.368±0.1	1	2.9	45
F	5 mg/L	1.03-0	0.352±0.4	0	0.0	-
Na	20 mg/l	76.5-0.1	14.13±11	7	20.6	26,33,36,37,52,53,56
K	12 mg/l	15.8-0.1	2.703±1.3	1	2.9	33

Table (2): Ranges, mean, and standard deviations of results compared to water quality guidelines in physical and biological examination.

Parameters	Normal range	Range of sample	Mean±SD	No. of sample out of range	Percent	Name of sample
Conductivity	800	4-1739	268±318	2	5.9	33, 45
pH	6.5-8.5	6.11-7.89	7.3647±0.3938	1	2.9	33
Turbidity	4	0.02-3.18	0.414±0.506	0	-	-
Seda	No	No		-	-	-
Color	Color less	Color less		-	-	-
Odor	Odor less	Odor less		-	-	-
Taste	Taste less	Taste less		-	-	-
E. coli	0	0	0	-	-	-
Coliform	0	0	0	-	-	-
Fungi	0	0	0	-	-	-

Table (3): Ranges, mean, and standard deviations of results compared to water quality guidelines in heavy metals.

Parameters	Normal range	Range of sample	Mean±SD	No. of sample out of range	Percent	Name of sample
Silver	50 µg/l	0.5-5.9	3.2±3.8	0	0.0	0.0
Aluminum	2 mg/L	0.2-57.2	4.4±12.2	7	20.6	27, 32, 37, 51, 52, 56, 63
Arsenic	0.1 mg/dl	0.2-6.4	1.5±2.1	8	23.5	26, 31, 33, 35, 36, 39, 61, 63
Boron	1 mg/L	2.5-884.6	151.1±232.4	15	44.1	
Barium	0.3 mg/L	0.2-379.5	36.4±90.1	16	47.1	

Beryllium	1 µg/l	0.00-9.2	1.2±0.0	1	2.9	33
Bismuth	1 µg/l	<0.1	<0.1	0	0.0	0
Bromine	10 µg/l	0.9-1702.3	108.1±349.5	15	44.1	
Cadmium	1 µg/l	<0.1	<0.1	0	0.0	0
Chromium	2µg/l	0.1-0.6	0.3±0.2	5	14.7	26, 31, 33, 35, 36
Cesium	1 µg/l	0-199.5	12.9±40.8	2	5.9	33, 62
Cupper	2 mg/L	0.1-1.4	0.4±0.5	6	17.6	
Mercury	<0.5 µg/l	<0.1	<0.1	0	0.0	0
Iodine	2.33	0.1-42.2	5.1±8.9	17	50.0	
Lithium	1 mg/L	0.2-4436.4	297.8±1144.9	16	47.1	
Manganese	0.5 mg/L	0.1-159.4	13.7±45.9	15	44.1	
Nickel	1 mg/L	0.2-4.9	1.6±1.2	10	29.4	
Lead	1 µg/l	<0.1	<0.1	0	0.0	0
Rubidium	1 µg/l	0.1-285.5	16.7±67.1	12	35.3	
Antimony	0.5 mg/L	0.5-1.3	0.9±0.4	12	35.3	
Selenium	<0.01 mg/l	0.2-1.9	0.5±0.6	16	47.1	
Tin	1 µg/l	<0.1	<0.1	0	0.0	0
Strontium	70 µg/L	0.1-485.2	95.3±118.5	16	47.1	
Tantalum	1 µg/l	<0.1	<0.1	0	0.0	0
Thallium	1 µg/l	<0.1	<0.1	0	0.0	0
Uranium	1 µg/l	0.2-1.9	0.7±0.6	10	29.4	
Zinc	1 µg/l	0.1-17.4	2.5±5.1	12	35.3	
Bicarbonate	400	7.0-647.0	111.6±137.0	2	5.9	33, 45

Appendix 1. The commercial name of the different sample used in this study.

26	Manao
27	Nuran
30	Elite
31	Evian
32	Berain
33	-----
34	Hamidiya
35	Highland
36	Nord Water
37	Safia
38	Tannourine
39	Volvic
40	Nestle
41	Ev ian Live
42	AQUA
43	Resorts World
44	BouKein
45	Vittel
46	Evian
47	alain
48	Watsons
49	Nongfu spring
51	manahl
52	healthy
53	DEEM
54	knuckles
55	Dasani
56	Moya
57	Viva
58	Wikins
59	Kinley
60	Sirma
61	damla
62	Nestle
63	pinarim
64	Jeema
68	-----

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