

**MICROBIOLOGICAL AND CHEMICAL CHARACTERISTICS OF POTABLE
BOTTLED WATER IN TANZANIA**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD
SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.
MOROGORO, TANZANIA.**

2017

EXTENDED ABSTRACT

Tanzania has experienced an increase in the consumption of bottled water as a result of the population building trust that it is safer than tap water. It is the interest of public to consume water of high quality both microbiologically and chemically. Due to the fact that bottled water is produced from different geographical regions, it is expected to have different qualities in microbial flora loading, essential elements and heavy metals. The aim of this study was to assess the quality and safety of the bottled water produced from different geographically areas of Tanzania and make recommendations to the public and surveillance authorities.

A cross-sectional study in which a total of 15 brands of bottled drinking water manufactured in 10 Regions which constitute four geographical zones of Tanzania were analyzed for microbiological contamination using the membrane filtration method and reported in terms of MPN/100 mls. Also Total Plate Count was done and reported in cfu/ml. Analysis of the presence of *Cryptosporidium* oocysts used the concentration method which consisted of three stages:- concentration, separation and microscopic detection.

The Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) was used to determine the concentration of both essential macro elements (Calcium, Magnesium, Potassium and Sodium) and heavy metal (Lead, Mercury, Cadmium and Arsenic) and the results were expressed in milligram per litre.

Total coliform was detected in 2 brands of bottled drinking water from Lake Zone and Eastern Zone, also 10 brands of bottled drinking water from Northern Zone 3 out of 3,

Lake zone 3 out of 5, Eastern Zone 2 out of 5 and Southern Highland Zone 2 out of 2 had a total bacterial count loads of above 500 cfu/ml, Eastern zone and Lake Zone each had a bottled drinking water brand exceeding 3×10^5 . *Cryptosporidium* oocysts were not detected in all 15 bottled drinking water brands so complying with the standards.

The concentrations of all three elements Calcium, Magnesium and Sodium were very low and statistically significant at a 'p' value of 0.05 as compared to their recommended maximum values by TBS which are 250mg/L, 100mg/L and 200mg/L for Calcium, Magnesium and Sodium respectively. Potassium was not compared because up to this moment there is no recommended value provided by TBS/WHO.

Another objective was to compare the information displayed on the label if they match with the actual concentration in bottled drinking water. With exception of Magnesium which was statistically different at a 'p' value of 0.05, the remaining had no difference with their label. For heavy metals the results show that, the concentrations obtained in all samples were within the legal limits.

Microbiologically, all bottled drinking water brands were safe to drink and can be used to process other food. The consumers of bottled water should make sure they do not depend on water to get their essential elements, because it was observed to have very low concentration of macro elements. Other sources of food higher in these elements should be used. The manufacturers of water should make sure that they balance the lost minerals before water is packed for sale.

DECLARATION

I, Emmanuel Raphael do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is the result of my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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ACKNOWLEDGEMENTS

Above all I highly thank the Almighty God whose mercies have been upon me throughout my studies at SUA and I have thus reached this far. I am grateful to the Bukoba District Council (BDC) for giving me an opportunity to study.

My sincere appreciation goes to my supervisors Professor Bernard E. Chove and Professor Bernadette K. Ndabikunze who tirelessly guided me right from the time of proposal development up to the end of this study.

My special appreciations go to my family wife Angel Leonard, my two sons Gabriel and Innocent and my daughter Emmaculata. You all deserve a deep acknowledgement. Thanks for your unconditional love, care, support and encouragement you have given me throughout to a stage of accomplishing of my studies.

DEDICATION

This dissertation is dedicated to my father Raphael Haisule and my mother Regina Mbwiga for being my inspiration, and my brothers Stephen Raphael and Michael Raphael who devoted much of moral support.

TABLE OF CONTENTS

EXTENDED ABSTRACT	ii
DECLARATION.....	iv
COPYRIGHT	v
ACKNOWLEDGEMENTS.....	vi
DEDICATION.....	vii
TABLE OF CONTENTS.....	viii
LIST OF TABLES	xii
LIST OF ABBREVIATIONS AND ACRONYMS	xiii
CHAPTER ONE.....	1
1.0 INTRODUCTION.....	1
1.1 Microbiological Indicators for Drinking Water	2
1.1.1 Total coliforms	3
1.1.2 Thermotolerant (faecal) coliforms	3
1.1.3 Total plate count (TPC).....	3
1.2 The Process of Water Sanitation and Consumption of Bottled Water in Tanzania	4
1.3 Current Status of Water Borne Diseases	5
1.4 Heavy Metals.....	5
1.5 Other Essential Metals	6
1.6 Problem Statement and Justification of the Study	6
1.7 Objectives.....	7
1.7.1 Overall objective	7
1.7.1.1 Specific objectives.....	7
CHAPTER TWO.....	8
2.0 LITERATURE REVIEW.....	8

2.1 Water Sources Pollution.....	8
2.2 Microorganism in Potable Bottled Drinking Water	8
2.3 Presence of Heavy Metals	9
2.4 Presence of Other Essential Elements	11
2.5 List of Manuscripts.....	12
REFERENCES	13
CHAPTER THREE (PAPER 1)	20
Evaluation of Some Heavy Metals and Essential Elements Content of Potable Bottled Water Manufactured in Different Regions of Tanzania	20
ABSTRACT	21
Introduction	23
Objective of the Study.....	28
Material and Methods.....	28
Study area.....	28
Selection of samples.....	28
Sample size determination	28
Sample collection and handling	29
Quantification of lead, cadmium, mercury, arsenic, calcium, magnesium, sodium and potassium in sampled bottled drinking water.....	29
Standards, chemicals and certified reference material	30
Sample preparation.....	30
Statistical analysis	31
Results	31
Discussion.....	35
Conclusion.....	37
REFERENCES	38

CHAPTER FOUR (PAPER 2)	44
Microbiological Assessment of Potable Bottled Drinking Water Brands	
Manufactured in Different Regions of Tanzania Mainland	44
ABSTRACT	45
Introduction	46
Objective of the Study	48
Material and Methods	48
Study area	48
Selection of samples	49
Sample size determination	49
Sample collection and handling	49
Analysis of the Sampled Bottled Drinking Water for Total Plate Count (TPC),	
Total coliforms and fecal coliforms	50
Total plate count (viable count)	50
Calculation	50
Determination of total coliform count	51
Incubation	51
Confirmation test	51
Determination of fecal coliform by membrane filtration method	51
Presumptive test	52
Confirmation test	52
Quantification/isolation of <i>Cryptosporidium</i> oocysts in sampled bottled drinking	
water	52
Microscopic examination	53
Statistical Data Analysis	53
Results	53

Total Plate Count (TPC) for Total Heterotrophic Plate Count.....	53
Total coliforms	55
Discusion.....	55
Conclusion.....	56
REFERENCES	58
CHAPTER FIVE.....	63
5.0 CONCLUSION AND RECOMMENDATIONS	63
5.1 Conclusion.....	63
5.2 Recommendations	64

LIST OF TABLES

Table 2.1: Major categories of water pollutants.....	8
Table 2.2: Current drinking water quality guidelines ($\mu\text{g/L}$) for heavy metals	9
Table 2.3: Some human Diseases Transmitted by Polluted Water	10
Table 2.4: Amount of minerals to be contributed by water, assuming a daily intake of 2 litres, as percentage RDI, Recommended Daily Intake for adults (70kg average body weight).	11
Table 2.5: The upper limit for the aesthetic quality of drinking water	11
Table 3.1: TBS aesthetic quality standard of drinking water	24
Table 3.2: TBS/WHO upper limits for toxic substances in drinking water and their effect to human.....	27
Table 3.3: Minimum, Maximum and Median Concentration (Mg/L) of Different Parameters of Macro Elements at Different Sampling Zones.....	32
Table 3.4: The concentration of Heavy Metals in mg/L of different drinking bottled water brands	34
Table 3.5: The correlation between actual concentration and respective label of calcium, magnesium, potassium and sodium with their respective label and how they do correlate	35
Table 4.1: Total Plate Count (TPC) of heterotrophic microbes in sampled bottled drinking water	54
Table 4.2: Paired differences for TPC from TPC 500 (CFU/ML) by zones	54

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
CD4	Cluster of Differentiation 4
DNA	Deoxyribonucleic Acid
EAS	East African draft Standards
EC media	<i>Escherichia coli</i> media
ECE	European Commission Environment
FTP-CDW	Federal-Provincial-Territorial Committee on Drinking Water, Health Canada
GMP	Good Manufacturing Practice
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectroscopy
ISO	International Organization for Standardization
PCRWR	Pakistan Council of Research in Water
pH	Hydrogen potential
TBS	Tanzania Bureau of Standards
UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

It is reported that the global annual growth rate of bottled water consumption was 5.5% in the period between 2004 and 2009, and reached a consumption of 203 million tons of bottled water in 2009 (Alsulaili *et al.*, 2015). In recent years bottled water consumption in the world increases by an average of 7% each year (Kyaw *et al.*, 2015). In Tanzania, 0.3 percent of the households depend entirely on bottled drinking water (URT, 2014).

Packaged water is any potable water that is manufactured or processed for sale, which is sealed in food-grade bottles (Oluyeye *et al.*, 2014; Halage *et al.*, 2015). Safe water is the one which is free from chemical substances and micro-organisms in concentrations which could cause illness or body disorders in any form (EAS, 2009; WHO, 2011; Meki *et al.*, 2014). Despite the common belief that bottled water is safe to drink and has a better taste than tap water, scientific studies have shown that the belief is not necessarily true (Abed and Alwakee, 2007; Hu *et al.*, 2011; Halage *et al.*, 2015).

According to Tanzania Bureau of Standards (TBS) (2008); World Health Organization (WHO, 2011); East African Draft Standards (EAS, 2009) require total coliform, fecal coliform and *Escherichia coli* for drinking water to be zero.

Cryptosporidium is a small protozoan parasite that infects the microvillous region of epithelial cells in the digestive and respiratory tract of vertebrates. It is an obligate intracellular parasite of man and other mammals, birds, reptiles and fish. It requires its host to multiply. Environmentally robust oocysts are shed by infected hosts into the

environment. These oocysts can survive the adverse conditions on the environment for months until it is ingested by a new suitable host (WHO, 2006).

A heavy metal is any metal and metalloid element that has a relatively high density ranging from 3.5 to 7 gcm⁻³ and is toxic or poisonous at low concentrations, which includes mercury, cadmium, arsenic, chromium, thallium, zinc, nickel, copper and lead (Barti, 2012; Gautam *et al.*, 2014; Jaishankar *et al.*, 2014). Heavy metals are highly toxic and can cause damaging effects even at very low concentration (Chennaiah *et al.*, 2014). These metals can be found in water through the contamination of water with these heavy metals from the environment.

Mineral nutrients are the chemical elements required by living organisms, other than the four elements carbon, hydrogen, nitrogen, and oxygen, present in common organic molecules. Drinking water may contain some of the fourteen essential minerals (Khater *et al.*, 2014) and four macronutrients in water include Calcium, Potassium, Magnesium and Sodium (Abd El-Salam *et al.*, 2008).

1.1 Microbiological Indicators for Drinking Water

There are three groups of bacteria each of which is taken as an indicator of drinking water quality and each has a different level of risk. The groups are Total coliforms, Fecal coliforms and *E. coli*. In Tanzania, shallow wells and tap water have been reported to have high level of both total coliforms and fecal coliform (Saria *et al.*, 2011). Wells and Municipal water supplies are used as sources of raw materials in the processing of food beverages including potable bottled water.

1.1.1 Total coliforms

Total coliforms refer to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. The group includes thermotolerant coliforms and bacteria of faecal origin, as well as some bacteria that may be isolated from environmental sources (WHO, 2008), in drinking water no coliform bacteria is allowed (TBS, 2008).

1.1.2 Thermotolerant (faecal) coliforms

Faecal coliform denotes coliform organisms which grow at 44 - 44.5°C and ferment lactose to produce acid and gas. *Escherichia coli* are present in large numbers in the normal intestinal flora of humans and animals, where it causes no harm. However, in other parts of the body, *E. coli* can cause serious disease, such as urinary tract infections, bacteraemia and meningitis. More than 95 per cent of thermotolerant coliforms isolated from water are the gut organism *Escherichia coli* (WHO, 2003). According to Tanzania Bureau of Standards (TBS, 2008); World Health Organization (WHO, 2011) and East African Draft Standards (EAS, 2009) require total, faecal coliforms and *Escherichia coli* for drinking water to be zero.

1.1.3 Total plate count (TPC)

Total plate count or heterotrophic plate count include all pathogens and non pathogens and is used to determine the hygienic status of food produced (WHO, 2011). The total plate count is used to enumerate aerobic, mesophilic organisms that grow in aerobic conditions under moderate temperatures of 20 to 40°C. In Tanzania, there is no limit for total plate count but in Brazil, USA and Australia the upper limit is 500 cfu/ml, because TPC above 500 cfu/ml indicates the possibility of coliform bacteria (Diduch *et al.*, 2015). The study used the upper limit of 500 cfu/ml because the aim is the safety of drinking water, coliform being the indicator bacteria.

The heterotrophic bacteria may cause diseases when their population in drinking water are 10^8 - 10^9 cfu for *Pseudomonas aeruginosa*, $>10^8$ cfu for *Aeromonas hydrophila*, 10^4 - 10^7 cfu for *Mycobacterium avium* and 10^6 - 10^9 cfu for *Xanthomonas maltophilia*, respectively (Diduch *et al.*, 2015).

Protozoa uses *Cryptosporidium spp* as an indicator which is a genus of coccidian protozoan parasites, found worldwide in a variety of vertebrate hosts including human (WHO, 1996). *Cryptosporidium* is a waterborne protozoan pathogen which is a causative agent of gastro-intestinal illnesses in humans and animals (Swai and Schooman, 2010; Tellevik *et al.*, 2015). *Cryptosporidium* is the most persistent in the environment (WHO, 2009), most resistant to chemical disinfection (Edberg, 2013) and smallest in size (4-6) microns (Tyagi *et al.*, 2006) with double walls (Bouزيد *et al.*, 2013), so most difficult to remove by filtration (WHO, 2011). The presence of *Cryptosporidium* in water indicates a human and possible animal origin contamination (Ajeegah *et al.*, 2007).

1.2 The Process of Water Sanitation and Consumption of Bottled Water in Tanzania

Water sanitation is the key issue to prevent contamination of the environmental excreta and, therefore, prevents transmission of pathogens that originate in faeces of an infected person (Traerup *et al.*, 2011; Brown *et al.*, 2017). Scarce water resources and poor sanitation make Tanzania vulnerable to outbreaks of cholera and other waterborne diseases (Mboera *et al.*, 2011). Access to safe drinking water in towns is decreasing as 71.0 percent of the Tanzanian population had access to safe piped water in 2002 and in 2012 dropped to 59 percent (URT, 2014). This decline appears to be related to the phenomenon of rural to urban migration (URT, 2011). Bottled water may undergo treatment of filtration, decantation, chlorination and dechlorination, UV light radiation and ozonation prior to packaging (TBS, 2008). Sales of bottled water have increased dramatically in recent years

largely because of the public perception of purity and safety and public concern about the quality of tap water (Kassenga, 2007).

1.3 Current Status of Water Borne Diseases

Diseases caused by inadequate water, sanitation and hygiene result in 4.2 % of global deaths and 90.0 % of that burden is born by children under five years of age (Bartram and Cairncross, 2010). The global under five deaths in the year 2015 was 5 941 937 out of which diarrhoea contributed 525 977 (8.9%) (WHO/MCEE, 2015). In Tanzania, 9.0 % of all mortality in children under five years is due to diarrhoea (WHO, 2010). Tanzania the under five death in the year 2015 was 98 180 in which diarrhoea contributed 7 896 (8.0%) (WHO/MCEE, 2015). Human excreta can contain bacterial, viral, protozoan and helminthic pathogens, excreta-related infections travel through a variety of routes from one host to the next, either as a result of direct transmission through contaminated hands, or indirect transmission via contamination of drinking water, soil, utensils, food and flies (Brown *et al.*, 2017).

1.4 Heavy Metals

Heavy metals are among the common environmental pollutants, and their occurrence in waters and biota indicate the presence of natural or anthropogenic sources. Lead, cadmium, and arsenic have no important functions in human body rather play toxic role to living organism, hence are considered as toxic elements (Mohamed and Khamis, 2012). Heavy metals are toxic because they react with body's biomolecules, clog up receptor sites, break and bend sulphur bonds in important enzymes such as insulin, and damage the DNA. Toxic metals are usually present in industrial, municipal and urban runoff, which can be harmful to humans and biotic life (Mohod and Dhote, 2013). Guidelines for mercury, lead, arsenic and cadmium in drinking water quality are detailed in Table 2.2.

1.5 Other Essential Metals

Long term consumption of waters low in minerals (such as Calcium, Magnesium and Fluoride) may be responsible for important health problems such as osteoporosis, nephrolithiasis (kidney stones), colorectal cancer, hypertension and stroke, coronary artery disease, insulin resistance and obesity, endothelial dysfunction, increased vascular reactions (WHO, 2011; Mihayo and Mkoma, 2012). Water should not exceed 250 mg/L for Calcium, 200 mg/L for sodium and 100 mg/L for Magnesium (TBS, 2008).

Calcium causes unpleasant taste of water for some consumers, calcium taste threshold is about 100-300 mg/l, unpleasant taste starts from 500 mg/l, but it also depends upon the presence of other ions. The magnesium content exceeding 170 mg/l together with the presence of chloride and sulphate anions are responsible for the bitter taste of water (Rapant *et al.*, 2017).

1.6 Problem Statement and Justification of the Study

There is an increase of waterborne diseases reported countrywide as an alarming issue which needs intervention to assess the safety of bottled drinking water from different sources and come out with appropriate recommendations. Taking the case of ongoing cholera epidemic outbreak in Tanzania, which began in August 15, 2015 in Dare es Salaam to November 26, 2016 has affected 23 of 25 regions in mainland Tanzania with a cumulative case count of 23 258 (Narra *et al.*, 2017). The majority of cases and deaths have been reported from 23 regions in Tanzania. Drinking waterborne outbreaks have the capacity to result in the simultaneous infection of a large number of persons and potentially a high proportion of the community (WHO, 2008).

The Centers for Disease Control and Prevention (CDC) in 1994 as cited by Abd El-Salam *et al.* (2008), reported an outbreak of cholera in the United States of America associated with bottled water. In Portugal cholera outbreak occurred in the mid-1970s due to the use of bottled water from a contaminated limestone aquifer.

Abd El-Salam *et al.* (2008) found that 54.8% of the examined bottles were bacteriological unacceptable according to the Egyptian standards. In Bulawayo the local bottled water brands, had a 10% mean prevalence for total coliforms while *E. coli* and the fecal coliforms had a mean prevalence of 2.5% and 5% respectively (Moyo *et al.*, 2014). In India 10.5% of the tested bottled water samples were found to have coliform bacteria (Jeena, 2005).

1.7 Objectives

1.7.1 Overall objective

The overall objective of the study was to assess the extent of microbial and heavy metal contamination in commercially available bottled water, compliance of selected elements to National and International standards and associated risk factors of water borne diseases.

1.7.1.1 Specific objectives

- (i) To establish the presence, and the associated of total bacterial count, total and fecal coliforms in the sampled bottled water.
- (ii) To determine concentration of heavy metals in the sampled bottled water.
- (iii) To assess the compliance of the sampled bottled water on calcium, sodium, potassium and magnesium content, and authenticity of the labels on sampled bottled drinking water.
- (iv) To identify the presence of *Cryptosporidium* species in the sampled bottled water.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Water Sources Pollution

Water sources pollution occurs when pollutants are directly or indirectly discharged into water, then water pollution affects the community (Mann *et al.*, 2014).

Table 2.1: Major categories of water pollutants

Category	Example	Source
A Causes health problem		
1. Infectious agents	Bacteria, viruses, parasites	Human and Animal excreta
2. Organic chemicals	Pesticides, Plastics, Oil, gas, detergents	Industrial, household and farm use
3. Inorganic chemicals	Acids, caustics, salts, metals	Industrial effluents, household cleansers,
4. Radioactive material	Uranium, thorium, cesium, iodine, radon	Mining/processing ore, power plants, weapons, natural sources
B Causes ecosystem disruption		
1. Sediment	Soil, silt	Land erosion
2. Oxygen demanding wastes	Animal manure and plant residues	Sewage, agricultural runoff, paper mills, food processing
3. Thermal	Heat	Power plants, Industrial cooling

Sources: UNEP, 2010; Sasikaran *et al.*, 2012; Owa, 2013; Mann *et al.*, 2014.

2.2 Microorganism in Potable Bottled Drinking Water

There is a long list of human diseases related to drinking water. The failure to properly treat drinking water is responsible for many adverse and environmental effects. Bacteria

diseases include salmonellosis, shigellosis, cholera; viral diseases include infectious hepatitis and gastroenteritis; Protozoans and amoebas cause giardiasis, amoebic dysentery and cryptosporidiosis (Sekhara *et al.*, 2017).

2.3 Presence of Heavy Metals

The heavy metal toxicity in drinking water includes damaged or reduced mental and central nervous function and lower energy level. They also cause irregularity in blood composition, badly affect vital organs such as kidneys and liver. The long term exposure of these metals result in physical, muscular, neurological degenerative processes that cause Alzheimer's disease (brain disorder), Parkinson's disease (degenerative disease of the brain), muscular dystrophy (progressive skeletal muscle weakness) and a nervous system disease that affects brain and spinal cord (Vuai, 2012). Guidelines for drinking water quality are detailed in Table 2.2.

Table 2.2: Current drinking water quality guidelines ($\mu\text{g/L}$) for heavy metals

HEAVY METAL	TBS	WHO	USEPA	ECE	FTP -CDW	PCRWR	EAS
Arsenic	50	10	10	10	10	50	10
Cadmium	5	3	5	5	5	10	3
Mercury	1	6	2	1	1	1	1
Lead	50	10	15	10	10	50	10

Source: Tanzania Bureau of Standards (TBS, 2008); World Health Organization (WHO, 2011); East African Draft Standards (EAS, 2009).

Some human diseases that can be transmitted by polluted water are shown in Table 2.3.

Table 2.3: Some human Diseases Transmitted by Polluted Water

Disease	Infectious Agent	Type of Organism	Symptoms
Cholera	<i>Vibrio cholerae</i>	Bacterium	Severe diarrhea, vomiting, fluid loss
Dysentery	<i>Shigella dysenteiae</i>	Bacterium	Infection of the colon causes painful diarrhea with mucus and blood in stool, abdominal pain
Enteritis	<i>Clostridium perfringens</i>	Bacterium	Inflammation of the small intestine causes general discomfort, loss of appetite, abdominal cramps and diarrhea
Typhoid	<i>Salmonella typhi</i>	Bacterium	Headache, loss of energy, fever, a pink rash appears along with hemorrhaging in the intestines
Infectious hepatitis	Hepatitis virus A	Virus	Inflammation of liver causes jaundice, fever, headache, nausea, vomiting, severe loss of appetite, aching in the muscle occurs
Poliomyelitis	Poliovirus	Virus	Sore throat, fever, diarrhea and aching in limbs and back, paralysis and atrophy of muscles
Cryptosporidiosis	<i>Cryptosporidium sp</i>	Protozoon	Diarrhea and cramps that last up to 22 days
Amoebic dysentery	<i>Entamoeba histolytica</i>	Protozoon	Infection of the colon causes painful diarrhea with mucus and blood in the stool
Schistosomiasis	<i>Schistosoma sp.</i>	Fluke	Tropical disorder of the liver and bladder causes blood in urine, diarrhea, weakness, lack of energy

Source: WHO (2007)

2.4 Presence of Other Essential Elements

Long term consumption of waters low in minerals may be responsible for important health problems such as osteoporosis, nephrolithiasis, colorectal cancer, hypertension and stroke, coronary artery disease, insulin resistance, obesity and endothelial dysfunction (WHO, 2011; Mihayo and Mkoma, 2012). The amount of minerals believed to be supplied by water daily is shown in Table 2.4.

Table 2.4: Amount of minerals to be contributed by water, assuming a daily intake of 2 litres, as percentage RDI, Recommended Daily Intake for adults (70kg average body weight).

Water contribution to daily intake (percent)				
Macro-elements				
Source of water	Calcium(Ca)	Magnesium (Mg)	Sodium (Na)	Potassium (K)
Bottled water	2.7-72	0.2-62	0.1-65	0.03-13.4

Source: Rosborg *et al.* (2015)

The upper limit of water quality of different minerals is shown in Table 2.5.

Table 2.5: The upper limit for the aesthetic quality of drinking water

Serial Number	Characteristics	Upper limit level (mg/Litre)
1	Sodium (as Na)	200
2	Magnesium (as Mg)	100
3	Calcium (as CaCO ₃)	250
4	Potassium	Not established

Source: TBS (2008)

2.5 List of Manuscripts

- Paper 1: Evaluation of Some Heavy Metals and Essential Elements Content of Potable Bottled Water Manufactured in Different Regions of Tanzania.
- Paper 2: Microbiological Assessment of Potable Bottled Water Brands Manufactured in Different Regions of Tanzania Mainland.

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CHAPTER THREE (PAPER 1)

**Evaluation of Some Heavy Metals and Essential Elements Content of Potable Bottled
Water Manufactured in Different Regions of Tanzania**

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ABSTRACT

Tanzania has experienced the increase in the consumption of bottled water as a result of the increase in population trust that it is safer than tap water. Drinking water may contain some quantities of the fourteen essential minerals and four macronutrients which include calcium, potassium, magnesium and sodium. Tanzania mainland has a diversity of geographical altitude which makes water sources to have different composition of minerals. The aim of this study was to establish if the differences of water sources are reflected in bottled drinking water in terms of macro minerals and heavy metals and if the manufacturers comply with the standards set by the Tanzania Bureau of Standards (TBS). A cross sectional study was conducted in which 15 brands of bottled water out of 54 brands registered by TBS were sampled from ten Regions of Tanzania Mainland, comprising four Zones; Lake Zone, Northern Zone, Southern Highland Zone and Eastern Zone. The sampled bottled water was taken to Southern and Eastern African Mineral Centre (SEAMIC) laboratory for analysis employing the ICP-OES technique. Samples were not filtered but acidified with nitric acid to a pH < 2. ICP-OES method is a flame technique with a flame temperature in a range from 6000 to 10000 K. The intensity of this emission is indicative of the concentration of the element within the sample. Each brand had three samples and each sample was read three times and the mean was calculated and was taken to be the actual concentration of the respective metal (mercury, lead, cadmium, arsenic, calcium, magnesium, potassium and sodium). Results were statistically analyzed at $p < 0.05$ using the Statistical Package for Social Science (SPSS). The results showed that the concentrations of calcium, sodium and magnesium were very small and the difference was statistically significant at $p < 0.05$ as compared to their recommended value by TBS/WHO. Potassium was not compared because there is no TBS/WHO Standard

recommended to date. For Calcium the concentration ranged from <0.01 mg/L to 526.53 mg/L, Magnesium ranged from <0.01 to 2.57 mg/L and Sodium ranged from 0.67 mg/L to 28.10 mg/L. The recommended concentrations in mg/L by TBS/WHO are Calcium 250 mg/L, Magnesium 100 mg/L and Sodium 200 mg/L. The concentration of Calcium, Magnesium and Potassium indicated on the label for all sampled bottled drinking water was not different from the concentrations determined in this study. However, the concentration of Magnesium differed significantly at $p < 0.05$ with value obtained in the laboratory. For heavy metals the results show that the concentrations obtained were very small and within the legal concentration limits of not exceeding the recommended levels of Mercury (0.001 mg/L), Lead (0.05 mg/L), Arsenic (0.05 mg/L) and Cadmium (0.005 mg/L).

Introduction

Bottled water is considered as a food product and is also regulated by the Tanzania Bureau of Standards (TBS). All bottled water products must comply with TBS quality standards, labeling regulations and Good Manufacturing Practices (TBS, 2008). Drinking water is one of the essential needs for life; it contains different types of substances dissolved, such as minerals and other biological compound (Albertini *et al.*, 2007).

Drinking water containing Calcium, Magnesium and Potassium can be crucial in the prevention of magnesium deficiency as well as contributing significantly to the daily calcium and potassium intake for persons with Calcium or Potassium-deficient diets (Lekskulchai, 2015). The balance between sodium and potassium intake is very important, as excess Sodium intake can result in depletion of potassium levels. In magnesium deficiency, there is a failure to retain potassium in sufficient quantities, and an excess intake of Potassium can interfere with magnesium uptake (Albertini *et al.*, 2007).

Calcium is important for fetal growth, pregnancy and lactation. It is essential in our body for teeth and bones formation, blood coagulation, right functioning of our nervous system (Albertini *et al.*, 2007). Inadequate calcium intake is a risk factor for osteoporosis, coronary disease (Cuartas *et al.*, 2006) and calcium oxalate stone formation (Albertini *et al.*, 2007). The Dietary Reference Index (DRI) of Ca^{2+} is highest for adolescents (1 300 mg) and for the elderly (1,200 mg). Adult men and women 19 to 50 years of age require 1,000 mg of Ca^{2+} per day (Azoulay *et al.*, 2001).

Magnesium is a cofactor and activator of more than 300 enzymatic reactions including glycolysis, ATP metabolism, transport of elements such as Sodium, Potassium and

Calcium through membranes, synthesis of proteins and nucleic acids. Magnesium deficient increases risk to humans of developing various pathological, cardiac arrhythmia, preeclampsia in pregnant women (Pip, 2000). For Magnesium, the DRI has been set at 6 mg/kg per day in industrialized countries (Azoulay *et al.*, 2001).

Sodium is an electrolyte the body needs to maintain fluid/water balance and muscle contraction. When it is consumed in excess amounts it can cause the body to hold on to water, which makes the heart work harder to pump blood through the body (WHO, 2010). Reducing dietary intake of sodium can help reduce high blood pressure by decreasing fluid retention (USEPA, 2003). Concentrations of sodium in excess of 200 mg/litre gives rise to unacceptable taste (Azoulay *et al.*, 2001). Healthy adults require at least 500 mg of sodium per day and nutritional experts have set a maximum recommended intake of 2 400 to 3 000 mg of Na⁺ per day (WHO, 2008).

Potassium is a cofactor for many enzymes and is required for the secretion of insulin, creatinine phosphorylation, carbohydrate metabolism and protein synthesis (WHO, 2009). The use of high doses of potassium salt causes chest tightness, nausea, vomiting, diarrhoea, hyperkalaemia, shortness of breath and heart failure, however, the data are not considered adequate to derive an upper limit for intake (Albertini *et al.*, 2007).

The TBS quality of drinking water is shown in Table 3.1.

Table 3.1: TBS aesthetic quality standard of drinking water

Serial number	Characteristics	Upper limit level (mg/Litre)
1	Sodium (as Na)	200
2	Magnesium (as Mg)	100
3	Calcium (as CaCO ₃)	250
4	Potassium	Not established

Source: TBS, 2008; WHO, 2011

On the other hand, it is known that more than 3 000 toxic dumping sites around the world are found to affect as many as 2 000 million people living near the toxic dumping sites and the per capita generation of hazard waste doubled worldwide between 1990 and late 2 000 (WBG, 2016). The toxic waste including heavy metal have been excessively released into the environment (WHO, 2005; Hashim and Ismail, 2016) due to rapid industrialization, manufacture of fertilizers and to the high production of industrial waste (Mrutu *et al.*, 2013; Olafisoye *et al.*, 2013; Gautam *et al.*, 2014; Cobbina *et al.*, 2015).

In Tanzania, environmental wastewater discharge comes from industries including; food processing, tanneries, fertilizer and petroleum refining, also it is estimated that 25.5 percent of the households dump their solid waste into water sources, valleys, pit latrines or other drainage areas (Tanzania Bureau of Statistics, 2009). Soil represent a major sink for heavy metals, which can then enter the food chain via water, plants or leaching into groundwater (Olafisoye *et al.*, 2013; Tom *et al.*, 2014; Bolawa and Adelusi, 2017). Drinking water can be a possible source of human exposure to heavy metals (Bugeja and Shoemake, 2015). Monitoring of heavy metals such as lead in waters of Tanzania in general is recommended in view of the possible risks to the health of consumers (Mshana and Sekadende, 2014).

Heavy Metal is defined as elements having a specific density of more than 3.5g cm^{-3} to 7gcm^{-3} (Barti, 2012; Gautam *et al.*, 2014). The main threats to human health from heavy metal are associated with exposure to cadmium, lead, mercury and arsenic (David *et al.*, 2013; Luqueno *et al.*, 2013; Jaishankar *et al.*, 2014). Additionally, there are others 19 elements known as heavy metal: antimony, bismuth, cerium, chromium, cobalt, copper, gallium, gold, iron, manganese, nickel, platinum, silver, tellurium, thallium, tin, uranium,

vanadium and zinc (WHO, 2005). Small amounts of heavy metal are common in our environment and diet, even some of them are necessary for good health such as iron, zinc, manganese etc (Luqueno *et al.*, 2013). Heavy metals are highly toxic and can cause damaging effects even at very low concentration if consumed above the recommended levels (Chennaiah *et al.*, 2014).

Heavy metal toxicity can result in cancer (Sullivan and Leavey, 2016), brain damage or the reduction of mental processes and central nervous function (Tiimub *et al.*, 2015), lower energy levels, damage to DNA, alterations on the gene expression, skin, muscle, blood composition, lungs, kidneys (Albaji *et al.*, 2013), liver, heart and other vital organs for humans and other living organisms (Luquelo *et al.*, 2013). Exposure to lead is cumulative over time (Hashim and Awing, 2016). High concentrations of lead in the body can cause death or permanent damage to the central nervous system, the brain, and kidneys. This damage results in behaviour and learning problems (Olafisoye *et al.*, 2013), memory and concentration problems, high blood pressure (Ifenna and Chinedu, 2012), It causes hearing problems, headaches, slowed growth (Razak *et al.*, 2015), reproductive problems in men and women (Harouny *et al.*, 2009), digestive problems, muscle and joint pain (Chennaiah *et al.*, 2014).

In larger doses, cadmium can accumulate in the liver and kidneys with a biological half – life in the human body, ranging from 10 to 33years (Mohod and Dhote, 2013; Fakhri *et al.*, 2015), and can replace calcium in bones, leading to painful bone disorders and to a renal failure. The kidney is considered to be the critical target organ in humans chronically exposed to cadmium by ingestion (Chennaiah *et al.*, 2014).

Table 3.2: TBS/WHO upper limits for toxic substances in drinking water and their effect to human

Metal	Substance	Upper limit (mg/litre)
Arsenic (as As)	- Causes toxicological and carcinogenic effects	0.05
	- Causes melanosis, keratosis and hyperpigmentation in humans	
	- Genotoxicity through generation of reactive oxygen species and lipid peroxidation	
	- Immunotoxic	
	- Modulation of co-receptor expression	
Cadmium (as Cd)	- Cause serious damage to kidneys and bones in humans	0.005
	- Bronchitis, emphysema, anemia	
	- Acute effects in children	
Lead (as Pb)	- Toxic to humans, aquatic fauna and livestock	0.05
	- High doses cause metabolic poison	
	- Tiredness, irritability anemia and behavioral changes of children	
	- Hypertension and brain damage	
	- Phytotoxic	
Mercury (total as Hg)	- Poisonous	0.001
	- Causes mutagenic effects	
	- Disturbs the cholesterol	

Source: TBS, 2008; WHO, 2011; Mohod and Dhote; 2013; Gautam *et al.*, 2014;

Objective of the Study

The objective of the study was to assess the extent of heavy metal contamination and compliance of selected essential elements (calcium, magnesium, potassium and sodium) to National and International standards and associated risk factors of water borne diseases.

Material and Methods

Study area

Geographical zoning was done by dividing the country into four zones as follows, Eastern zone had Dar es Salaam, Pwani and Morogoro Regions, Northern highland Zone had Arusha, Singida and Kilimanjaro Regions, Lake Zone had Kagera, Shinyanga and Mwanza Regions, Southern Highlands Zone had Iringa and Mbeya regions.

Selection of samples

In each region, the brands of potable bottled drinking water registered by TBS were listed down and a random sampling was done.

Sample size determination

The sample size was determined using formula of a known population described by (Kothari, 2009).

$$n = N \times Z^2 \times SD^2 / (N-1) \times d^2 + Z^2 \times SD^2,$$

Where n = estimated sample size,

Z = student's t value for an expected confidence interval (1.96),

SD = Standard deviation (0.1),

d = selected accepted errors (0.05),

N= Known population (The total numbers of registered by TBS potable bottled water in Tanzania are 54). Using the above formula the calculated sample size is 12 brands. I added 3 brands of bottled drinking water in order to make the sample size of 15 which will be a good representative of the Zones.

Sample collection and handling

A total of 15 brands of commercially available bottled drinking water were purchased randomly from different supermarket, shops and street vendors in four zones of the country. To keep the brand names anonymous, the samples were coded as A, B, C, D, E, F, G, H, J, K, L, M, N, P and R used throughout the study. All brands were sold in 1,500 ml plastic bottles which were sealed with plastic screw caps. Drinking water bottles obtained were from the same production year. As most consumers purchased drinking bottled water from supermarkets, shops and street vendors, these sources were preferred for analysis. The purchasing of samples took 48 hours from all the four Zones and were taken to the laboratory for analysis immediately after collection.

Quantification of lead, cadmium, mercury, arsenic, calcium, magnesium, sodium and potassium in sampled bottled drinking water

The measurements were performed using the PerkinElmer® Optima™ 7300 DV ICP-OES instrument (Connecticut, USA) equipped with WinLab32™ for ICP Version 4.0 software for simultaneous measurement of all analyte wavelengths of Calcium, Magnesium, Potassium, Sodium, Arsenic, Cadmium, Lead and Mercury. The Optima 7300 DV ICP-OES measured all wavelengths simultaneously.

The sample introduction unit employed included a cyclonic spray chamber and MEINHARD® concentric glass nebulizer. The concentric nebulizer provides excellent sensitivity and precision for aqueous solutions and samples with few dissolved solids (less than 1%), and is hence well suited for the analysis of water samples. The cyclonic spray chamber ensures high sample transfers to the plasma and fast rinse-in and rinse-out times, which improves productivity.

Standards, chemicals and certified reference material

PerkinElmer NIST® traceable quality control standards for ICP, Part No. N9300281 (for, Ca, Cd, Mg, Hg, As and Pb) and Part No. N9300280 (for K and Na) in 5% HNO₃, were used as the stock standards for preparing working standards. A total of four standards were used for calibration and were prepared from the two stock standards so that each metal ion will have two standards. ASTM® type I water (from a Millipore® filtration system, Millipore® Corporation, Massachusetts, USA) acidified with Suprapur® nitric acid (Merck®, Germany) was used as the calibration blank and for all dilutions. After constructing the calibration curves which were linear with a correlation coefficient of at least 0.9999, two Quality Control (QC) samples [one being a continuing calibration blank (CCB)], were run to monitor the instrument performance and evaluate longterm stability. Working standards were prepared by serial volume/volume dilution in polypropylene vials (Sarstedt®, Germany). Micropipettes (Eppendorf®, Germany) with disposable tips were used for pipetting solutions. NIST® certified drinking water reference material CRM 1643e was used for validating the developed method.

Sample preparation

The bottled waters were each poured into 50.0 mL graduated polypropylene vials and 500 µL of nitric acid was added. Spiked samples were similarly prepared with the addition of

known quantities of analyte ions from the stock standard solution. For drinking water compliance monitoring, a “total” element determination (dissolved plus suspended) is required. For this total determination of trace and matrix elements in drinking water, samples are not filtered, but acidified with nitric acid to a $\text{pH} < 2$.

Wavelength selection followed U.S. EPA method 200.7 and ISO regulation 11885. The reproducibility of the measurement was below 1%. The validity of the calibration was monitored by the Quality Control Check module within WinLab32 for ICP software. The quality control check standards were run at selected intervals in an unattended automated analysis run to ensure that the instrument performance remained consistent over the length of the analysis (Sarojam, 2010).

Statistical analysis

Minimum, maximum and median were computed using Statistical Package for Social Sciences (SPSS) version 20 software. The national and international standards used for studied bottled drinking water samples was compared using a t-test while the comparison among zones was done by Mann whitney U test. A test for differences between means was computed by Duncan’s Multiple Range Test at ($p < 0.05$).

Results

Table 3.3 summarizes the minimum, maximum and median concentrations in mg/l for Calcium, Magnesium, Potassium and Sodium. The results are summarized according to the Zones of production.

Table 3.3: Minimum, Maximum and Median Concentration (Mg/L) of Different Parameters of Macro Elements at Different Sampling Zones

ZONES	Country			Lake Zone			Northern Zone			Eastern Zone			Southern Zone		
Parameter	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med
Calcium	<0.01	526.53	21.63	9.36	113.5	14.9	<0.01	199.32	156.65	4.67	526.53	64.45	<0.01	21.63	10.82
Magnesium	<0.01	2.57	0.22	<0.01	1.81	0.41	<0.01	2.57	<0.01	0.04	2.25	0.24	<0.01	0.19	0.10
Potassium	<0.01	6.73	1.43	0.9	3.00	1.43	<0.01	6.73	5.00	0.7	3.36	1.97	<0.01	<0.01	<0.01
Sodium	0.67	28.10	6.93	2.27	25.41	2.27	0.67	22.00	16.47	1.02	28.10	18.32	6.25	7.43	6.84

The concentration of calcium ranged from < 0.01 mg/L to 526.53 mg/L with a median of 21.63 mg/L, with exception of Brand B from Eastern Zone which exceeded the recommended upper limit of TBS by having a concentration of 526.53 mg/L all the 14 drinking water brands had the concentration below the set TBS upper limit. Magnesium had a concentration ranging from < 0.01 mg/L to 2.57 mg/L with a median of 0.22 mg/L. All the 15 brands were within the TBS upper limit. Sodium concentration ranged from 0.67 mg/L to 28.10 mg/L with a median of 6.93 mg/L, all the 15 drinking water brands were below the set upper limit standard by TBS. Generally the concentrations of all three elements were significantly very low as compared to their recommended value by TBS. Potassium were not compared because up to this moment there is no recommended value for it.

All the elements Calcium, Magnesium and Sodium were within the recommended upper limit standard set by TBS/WHO with exception of brand B from Eastern Zone which exceeded the recommended value for calcium.

Table 3.4: The concentration of Heavy Metals in mg/L of different drinking bottled water brands

Sample code	Mercury	Lead	Cadmium	Arsenic
A	< 0.001	< 0.001	< 0.001	< 0.001
B	< 0.001	< 0.001	< 0.001	< 0.001
C	< 0.001	< 0.001	< 0.001	< 0.001
D	< 0.001	< 0.001	< 0.001	< 0.001
E	< 0.001	< 0.001	< 0.001	< 0.001
F	< 0.001	< 0.001	< 0.001	< 0.001
G	< 0.001	< 0.001	< 0.001	< 0.001
H	< 0.001	< 0.001	< 0.001	< 0.001
J	< 0.001	< 0.001	< 0.001	< 0.001
K	< 0.001	< 0.001	< 0.001	< 0.001
L	< 0.001	< 0.001	< 0.001	< 0.001
M	< 0.001	< 0.001	< 0.001	< 0.001
N	< 0.001	< 0.001	< 0.001	< 0.001
P	< 0.001	< 0.001	< 0.001	< 0.001
R	< 0.001	< 0.001	< 0.001	< 0.001

The concentration of all the four analyzed heavy metals, that is mercury, lead, cadmium and arsenic was very low and was almost the same for all the samples collected from different regions in mainland Tanzania.

Table 3.5 show that calcium concentration was not statistically different between the actual concentrations with its labeled concentrations although the correlation was weak. For magnesium actual concentration was significantly different from the labeled one at a 'p' value of 0.05, the label shows a higher value compared to the actual concentration of Magnesium in the bottled drinking water. Potassium showed no statistical difference between the actual concentrations and the label with a moderate correlation. The same was

for Sodium that the concentration of the content in the bottle was not statistically different from the concentration of the label. With exception of magnesium which was different from the respective label, other three essential elements calcium, potassium and sodium concentration was the same as those in their respective labels.

Table 3.5: The correlation between actual concentration and respective label of calcium, magnesium, potassium and sodium with their respective label and how they do correlate

Water Mineral	Calcium	Magnesium	Potassium	Sodium
Correlation	Weak correlation	Weak correlation	Moderate correlation	Moderate correlation
Correlation coefficient (r)	0.18	-0.309	0.608	0.575
Number of bottles labeled	11	11	12	13

Discussion

This study has shown that the quality of potable bottled drinking water as regards to heavy metals in the Tanzania mainland is satisfactory, and the concentrations obtained were within the legal limits. Similar results were obtained by David *et al.* (2013) when they investigated heavy metals in drinking water (sachet and bottled) in Nigeria. In that study, cadmium and lead were not present at all and their absence was not posing any health implication to the residents of the study area.

From the findings of this study, the manufacturers of sampled brands bottled drinking water conform to the stipulated standards. This may be due to the good selection of the sources of water to be used in processing of drinking water. Also, it might be due to routine monitoring of water source as recommended by Nazir *et al.* (2015) when he was

doing the study on the heavy metal concentration for drinking in Tanda dam Ethiopia, he recommended that potable water sources in the area should be routinely monitored to ascertain its suitability for drinking and other purposes, and Albaji *et al.* (2013) suggested that regular monitoring of heavy metals in drinking water and also food items should be performed in order to prevent excessive buildup of these heavy metals in the human food chain (Albaji *et al.*, 2013). In general the source of drinking water influences the suitability of water for drinking (URT, 2014).

According to TBS upper limit, all the samples were conforming with the standard because there is only upper limit and there is no lower limit. All of the brands tested, for essential elements in bottled drinking water, were deficient in essential minerals. The sampled bottled drinking water was deficient in the concentration of essential minerals (magnesium, calcium, sodium and potassium) which were present in low concentrations. The results of this study is the same as those obtained by Aris *et al.* (2012) when they were looking for concentration of ions in bottled water samples sold in Malaysia. Their findings indicated that calcium, magnesium and potassium were low in concentrations. This low concentration was due to treatment because in the source the ions were higher compared to bottled water (Alam *et al.*, 2017).

One brand of bottled water exceeded the recommended concentration of calcium which is 250 mg/l. Brand B had a concentration of 526.53 mg/l. Brands D, F and L had a concentration approaching the recommended value. All the remaining water brands had negligible concentrations of calcium.

Comparing with the label, three elements; calcium, potassium and sodium had the same mean with what have been claimed on the label where as magnesium was statistically different with p value 0.041. Other bottled samples did not show any concentrations of

some of the essential metal elements. For calcium and magnesium only 11 brands out of 15 sample brands was labeled, 12 brands out of 15 sampled brands for potassium was labeled and 13 brands out of 15 brands was labeled for sodium. Labeling is the requirement from TBS and is very important for the consumer to decide which brand of drinking water to buy. The number and type of parameters reported on the labels of bottled water showed a lack of homogeneity. In general, the concentration of species measured in this study was comparable to or slightly lower than values reported on the labels.

Conclusion

The chemical characteristic which involves major essential elements (calcium, magnesium, potassium and sodium) of the brands studied was extremely low irrespective of the factors such as the natural environment (geological setting, climate and topography). Those who are using bottled drinking water should also use other sources of these elements such as foods with high amounts of calcium, magnesium, potassium and sodium. The manufacturers of bottled water should consider recovering some lost essential elements during production in order to make water contribute such elements to enrich the products for the benefit of their consumers.

Another goal of this research work was to assess the concentration of some toxic heavy metals (lead, mercury, cadmium and arsenic). The results show that the concentration of all the drinking water samples was within the permissible limits set by TBS.

The number and type of parameters reported on the labels of bottled water showed a lack of homogeneity. Basic parameters (major ions) were usually indicated, whereas, for some brands generalized parameters were observed. In general, the concentration of species measured in this study was comparable to or slightly lower than values reported on the labels.

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CHAPTER FOUR (PAPER 2)

Microbiological Assessment of Potable Bottled Drinking Water Brands

Manufactured in Different Regions of Tanzania Mainland

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ABSTRACT

The consumption of bottled drinking water has significantly increased in Tanzania so that it is in the interest of public health to determine the microbiological quality of some brands of Tanzanian bottled drinking water manufactured in different Regions of the country.

A cross-sectional study in which a total of 15 brands of bottled drinking water manufactured in 10 regions which constitute four geographical zones of Tanzania were analyzed for total and faecal coliform using membrane filtration method and reported in MPN/100 mL, Total Plate Count (TPC) was analyzed using spread plate method and reported in cfu/ml. Analysis for the presence of *Cryptosporidium* oocysts using concentration method consisted of three stages: - concentration, separation and microscopic detection. Total coliform was detected in 2 brands out of 15 brands, also 10 brands out of 15 had a microbial loads (TPC) exceeding 500 cfu/ml. For the Eastern and Lake Zones each had one brand out of five exceeding 3×10^5 . *Cryptosporidium* oocysts were not detected in all 15 bottled drinking water brands. The results show that some of the water sources are contaminated with the surrounding environment. The Government and other stakeholders should consider intensifying surveillance activities and enforcing strict hygienic measures in the water sources as it can cause waterborne diseases problems to the consumers.

Introduction

Packaged drinking water is the water filled into hermetically sealed containers and suitable for direct consumption without necessary further treatment. It may undergo treatment of filtration, decantation, chlorination and dechlorination, UV light radiation and ozonation prior to packaging (TBS, 2008). Recently, there has been a considerable worldwide increase in the consumption of bottled water due to consumer's awareness regarding bottled water as a healthy and safe alternative to tap water (Kihupi *et al.*, 2015; Pant *et al.*, 2016). In Tanzania 0.3 percent of the household depends entirely on bottled drinking water (URT, 2014). Bottled water is not necessarily safe, as several studies show that there is presence of heterotrophic bacteria along with coliforms in bottled water in counts, exceeding national and international standards (Jeena *et al.*, 2005; Abd El-salam *et al.*, 2008; Sasikaran *et al.*, 2012).

Salmonella spp., *V. cholera*, *Cryptosporidium spp.* and *Escherichia coli*, which are frequently found in contaminated water are classified as zoonotic agents (Haupt *et al.*, 2005; WHO, 2009; Parsons *et al.*, 2015). Increasing number of waterborne outbreaks of cryptosporidiosis has been reported in Tanzania both in animals and human beings (Tellevik *et al.*, 2015).

Drinking water which is faecally contaminated, bacterial and protozoan pathogens are likely to be widely and rapidly dispersed (NRMMC, 2011). Kassenga, (2007), reported that in the city of Dar es salaam where 37% of bottled drinking water is produced had a prevalence of Total coliform bacteria and faecal coliform bacteria of 4.6% and 3.6%, respectively.

Some of the pathogens that are known to be transmitted through contaminated drinking-water lead to severe and sometimes life-threatening disease. Examples include typhoid, cholera, infectious hepatitis (caused by hepatitis A virus or hepatitis E virus) and disease caused by *Shigella* spp. and *E. coli* O157 (WHO, 2011). The Heterotrophic bacteria may cause diseases when their population in drinking water are 10^8 - 10^9 cfu for *Pseudomonas aeruginosa*, $>10^8$ cfu for *Aeromonas hydrophila*, 10^4 - 10^7 cfu for *Mycobacterium avium* and 10^6 - 10^9 cfu for *Xanthomonas maltophilia*, respectively (Diduch *et al.*, 2015).

In order to select a reference pathogen for any group, waterborne outbreaks indicated which pathogens have been able to break through the multiple barriers and cause disease also the prevalence and severity of the illness they cause, and the difficulty to control them through water treatment (Tyagi *et al.*, 2006; Ajeegah, *et al.*, 2007; WHO, 2011).

Heterotrophs are broadly defined as microorganisms that require organic carbon for growth including bacteria, yeasts and moulds which derive nutrients from the water body and/or materials in contact with water (WHO, 2002). The high total heterotrophic microorganisms load in bottled drinking water is resulting from poor source of water, poor hygienic practices during processing and care in the bottling plant (Jeena, 2005). The Total Plate Count (TPC) value exceeding 500 cfu/mL directly influences the determination of bacteria from the Coliform group, the most important routine determination qualifying water as safe in the context of microbiological indices (Diduch *et al.*, 2015).

Faecal contamination of groundwater or surface waters, as well as insufficiently treated and inadequately disinfected drinking-water, is the main causes of epidemic waterborne outbreaks caused by *Salmonella* spp (WHO, 1996).

All bottled drinking water produced or offered for sale within Tanzania is required to comply with the regulations and principles specified by Tanzania Drinking Water Quality Standards established by Tanzania Bureau of Standards which require total and faecal coliform to be zero per every 100 mls (TBS, 2008; WHO, 2011). For total plate count/Heterotrophic plate count must not exceed 500cfu/mL (WHO, 2003). The inoculums of cryptosporidium oocysts required to infect human lies between 10 – 100 cysts (Wallis *et al.*, 1996). Then the post treatment of drinking water should achieve a < 1 oocyst per 10 litres (WHO, 2011). In children Cryptosporidiosis causes retarded growth, which even if a child is treated the retarded growth continues (Perz *et al.*, 1998).

Cryptosporidium is a major enteric pathogen of patients with acquired immunodeficiency syndrome (AIDS), with infection rates of 53% reported among Tanzania AIDS patients with diarrhea while in general among HIV and non HIV only 17.3% of the population are infected by *Cryptosporidium* (Haupt *et al.*, 2005).

Objective of the Study

The objective of the study was to assess the safety of different brands of bottled drinking water in terms of presence of Total Plate count, total coliform, fecal coliforms and *Cryptosporidium* oocysts.

Material and Methods

Study area

Geographical zoning was done by dividing the country into four zones as follows, Coastal zone had Dar es Salaam, Pwani and Morogoro Regions, Northern highland Zone had Arusha, Singida and Kilimanjaro Regions, Lake Zone had Kagera, Shinyanga and Mwanza Regions, Southern Highlands Zone had Iringa and Mbeya Regions.

Selection of samples

In each region, the brands of bottled drinking water registered by TBS were listed down and a random sampling was done.

Sample size determination

The sample size is determined using formula of a known population described by (Kothari, 2009).

$$n = N \times Z^2 \times SD^2 / (N-1) \times d^2 + Z^2 \times SD^2,$$

Where n = Estimated sample size,

Z = Student's t value for an expected confidence interval (1.96),

SD = Standard deviation (0.1),

d = Selected accepted errors (0.05),

N= Known population (The total numbers of registered by TBS potable bottled water in Tanzania are 54). Using the above formula the calculated sample size is 12 brands. I added 3 brands of bottled drinking water in order to make the sample size of 15 which will be a good representative of the Zones.

Sample collection and handling

A total of 15 brands of commercially available bottled drinking water were purchased randomly from different supermarket, shops and street vendors in four zones of the country. To keep the brand names anonymous, the samples were coded as A, B, C, D, E, F, G, H, J, K, L, M, N, P and R used throughout the study. All brands were sold in 1,500 ml plastic bottles which were sealed with plastic screw caps. Drinking water bottles obtained were from the same production year. As most consumers purchase drinking

bottled water from supermarket, shops and street vendors these sources were preferred for analysis. The purchasing of samples took 48 hours from all the four Zones and was taken to the laboratory for analysis immediately after collection.

Analysis of the Sampled Bottled Drinking Water for Total Plate Count (TPC), Total coliforms and fecal coliforms

Total plate count (viable count)

The method is pour plate count. In which each dilution, 1 ml was pipetted into a separate, duplicate, appropriately marked petri plates. To each plate 20 ml of plate count agar was added within 15 minutes of the original dilution. Immediately, sample dilutions were mixed with agar medium thoroughly and uniformly. The agar was allowed to solidify; petri plates were inverted and incubated promptly for 48 ± 2 hours at 37°C . After incubation, the number of colonies on a dilution plate showing between 30 and 300 colonies was determined. A plate having 30-300 colonies was chosen because this range was considered significant. If there were less than 30 colonies on the plate, small errors in dilution technique or the presence of a few contaminants will have a drastic effect on the final count. Likewise, if there were more than 300 colonies on the plate, there will be poor isolation and colonies will have grown together (Bhandare *et al.*, 2009).

Calculation

To determine the number of cfu per gram of sample, the number of colonies (on a plate having 30-300 colonies) was multiplied by the number of times the original ml of bacteria was diluted (the dilution factor of the plate counted). Therefore, the number of cfu per ml in the original sample was found by multiplying the number of colonies (30-300 plate) x the dilution factor of the plate counted.

Determination of total coliform count

Incubation

The method used was a membrane filtration in which a volume of 10 ml portion of each original sample was transferred into five tubes containing Double strength of Mac Conkey broth. The tubes were incubated for 48 ± 2 hours at 37°C . These tubes were examined for gas production every 24 ± 2 hours, while a volume of 1 ml and 0.1 ml portion of each dilution was transferred into five tubes containing Single strength of Mac Conkey broth each separately. The negative tubes were re-incubated for additional 24 hours where a second time examination of gas production was conducted (Bhandare *et al.*, 2009).

Confirmation test

Confirmation test for total coliform was carried out for each gassing strength of Mac Conkey tube was gently agitated and loopful of suspension was transferred into tubes of Lauryl Tryptose broth and incubated for 48 ± 2 hours at 45°C . Examination of gas production was recorded. The calculation of Most Probable Number (MPN) of total coliforms based on combination of confirmed gassing Lauryl Tryptose broth tubes for three consecutive dilutions was done using MPN index Table and 95% confidence limits (Bhandare *et al.*, 2009).

Determination of faecal coliform by membrane filtration method

A volume of 10 ml portion of each original sample was transferred into five tubes containing Double strength of Mac Conkey broth. The tubes were incubated for 48 ± 2 hours at 37°C . These tubes were examined for gas production every 24 ± 2 hours, while a volume of 1 ml and 0.1 ml portion of each dilution was transferred into five tubes containing Single strength of MacConkey broth each separately. The negative tubes were

re-incubated for additional 24 hours where a second time examination of gas production was conducted (Bhandare *et al.*, 2009).

Presumptive test

Confirmation test for total coliform was carried out for each gassing strength of MacConkey tube was gently agitated and loopful of suspension was transferred into tubes of Lauryl Tryptose broth and incubated for 48 ± 2 hours at 45°C . Examination of gas production was recorded (Bhandare *et al.*, 2009).

Confirmation test

The gassing Lauryl Tryptose Broth tubes were transferred into EC medium and incubated for 48 ± 2 hours at 45°C . Then examination of gas production was recorded. The calculation of Most Probable Number (MPN) of fecal coliforms based on combination of confirmed gassing EC medium tubes for three consecutive dilutions was done using MPN index Table and 95% confidence limits (Bhandare *et al.*, 2009).

Quantification/isolation of *Cryptosporidium* oocysts in sampled bottled drinking water

The analysis of water samples for the presence of *Cryptosporidium* oocysts by concentration method consisted of three stages:- concentration, separation and detection (Clayton, 2011). 50 mls of the sample was concentrated into a single pellet by centrifugation at 3000 revolution per minute (rpm) for 10 minutes at a temperature of 4°C by using a 275 ml capacity swinging bucket rotor (model HS-4) and a Sorval RC-5B refrigerated superspeed centrifuge (DuPont Co., Wilmington, Del). Then the centrifuged sample was supernatant decanted to form a thick smear from deposit, air dried and

stained by modified Ziehl-Neelsen acid fast stain (hot method) stain and counter stained by 0.5% malachite green. It was washed in running tap water and air dried.

Microscopic examination

The presence of sporozoites or a densely packed cytoplasm within the oocysts was observed under objective microscope x 100 magnification (Rafiei *et al.*, 2014).

Statistical Data Analysis

Statistical data analysis was computed using statistical package for social sciences (SPSS) version 20 software. The national and international standards used for sampled bottled drinking water were compared using a t-test in which mean and standard deviation were calculated while the comparison among zones to see which zone had high contamination were done by one way ANOVA. A test for differences between means among zones was computed by Duncan's Multiple Range Test at ($p < 0.05$).

Results

Total Plate Count (TPC) for Total Heterotrophic Plate Count

The classification of water samples according to the total plate count load is given in Table 4.1. Results revealed that 10 out of 15 bottles of the sampled bottled drinking water had microbial loads above the 500cfu/ml. The microbial load varied from less than 10cfu/ml to 3×10^5 cfu/ml.

Table 4.1: Total Plate Count (TPC) of heterotrophic microbes in sampled bottled drinking water

S/no	Sample code	Total plate count (cfu/ml)
1	A	4.7×10^2
2	B	$>3 \times 10^5$
3	C	$< 1 \times 10^1$
4	D	7.1×10^2
5	E	1.2×10^2
6	F	5.9×10^2
7	G	6.6×10^2
8	H	1.63×10^3
9	J	$< 1 \times 10^1$
10	K	$< 1 \times 10^1$
11	L	$>3 \times 10^5$
12	M	1.48×10^3
13	N	9.7×10^2
14	P	1.29×10^3
15	R	7.4×10^2

Correlation between total plate count and total coliforms was tested using Pearson correlation (Minitab software). Pearson correlation ($r = 0.667$) was significant at $p < 0.05$.

Table 4.2: Paired differences for TPC from TPC 500 (CFU/ML) by zones

Zones	Minimum number of cells (CFU/ML)	Maximum number of cells (CFU/ML)	Median number of cells (CFU/ML)
Lake zone	0	300 000	660
Southern highland zone	740	970	855
Northern zone	590	1 290	710
Eastern zone	0	300 000	470

The microbial load by TPC in relation to the zone show that the difference in pollution was there but is not statistically significant at $p > 0.05$. Lake Zone and Eastern zone both had one brand exceeding 3×10^5 cfu which made the zones to be highly polluted.

Total coliforms

Tanzania Bureau of Standards/World Health Organization requires zero bacterial cells per 100 mls for total coliforms in bottled drinking water. Two brands of sampled bottled drinking water out of 15 brands did not meet the existing standards. Faecal coliforms were not detected in all sampled bottled drinking water tested in this study.

Lake Zone and Eastern zone had the same level of total coliforms, i.e one brand out of five selected had the total coliform. Two zones (Southern zone and Northern zone) had no total coliform bacteria. No *cryptosporidium* oocyst was detected in all 15 samples examined.

Discussion

Although TPC is a potential indicator of overall sanitation in bottling and water source; it may be harmless, but in some cases may indicate presence of infectious bacteria (Kassenga, 2007). TPC if it grows to large quantities has a tendency of changing the organoleptic properties of water (Diduch, 2015). Consumption of water with this type of microorganisms has no risk of diseases to normal person but it can pose a health risk to immunocompromised people and children (WHO, 2006; WHO, 2011).

Total coliforms obtained in two brands of sampled water differed from that obtained by Kihupi *et al.*, (2015) for which 4.6% were for both total and faecal coliform bacteria. In Bulawayo Moyo *et al.*, (2014) reported that 10% of analyzed samples had total coliform whereas *E. coli* and faecal coliforms had a mean prevalence of 2.5 and 5%, respectively. Kyaw *et al.* (2015) found 32.6% of bottled water sold in Yangon city failed to conform to the requirement of 0 total coliform per 100 ml. These results agree with the work of Halage *et al.* (2015) who reported that from packed water sold at Kampala outskirts, which

was contaminated with total coliform but not fecal coliform. Also, Meki *et al.* (2014) found that 8.9% of the bottled water sold in the Lusaka District had both total and faecal coliforms.

Since there was no faecal coliforms and *Escherichia coli*, the presence of total coliform indicates that water is contaminated by potentially environmental matters and not faecal materials. Total coliform organisms may not always be directly related to the presence of fecal contamination or pathogens in drinking water (WHO, 2008). These results revealed the danger of waterborne diseases in case of faecal contamination at the water source.

According to the Standard for *Cryptosporidium*, all the 15 samples conformed to the set standards. The results showed that there was no faecal contamination at the sources of bottled water and the environment surrounding the source of water because oocysts can resist water treatment, as reported by (Wallis *et al.*, 1996; Tian *et al.*, 2011).from Canada who observed *Cryptosporidium* oocysts positive rate of 3.5%, even after filtration.

Conclusion

Based on the microbiological quality of assessed water samples, the study has attempted to examine the public health implication of packaged bottled water products in Tanzania. Most packaged bottled water apparently was of good quality, faecal coliform and *E. coli* were not detected, only total coliform were detected, which indicated that two brands of bottled potable water were contaminated with environmental microorganisms and not faecal material.

The presence of high number of TPC is an indication that the sources of water were contaminated with environmental pathogens but water treatments were sufficient to eliminate the coliform bacteria with exception of two brands, which indicated failure in the treatment. It is recommended that the sources should be well managed and frequently monitored.

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CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The high amount of viable total bacterial count narrates that either the source of water is contaminated or the good manufacturing practices is not followed properly. The presence of total coliform is an indication that in the two brands of bottled drinking water; the source is contaminated and the disinfection practices in the processing is not capable of disinfecting the pathogens that might enter the water source. The absence of faecal coliform, *Escherichia Coli* and *Cryptosporidium* oocysts indicates that all 15 sampled brands of bottle drinking water were not contaminated by faeces, hence can not cause any health risk to consumers of respective bottled drinking water.

The chemical characteristic which involves major essential elements of the brands studied were extremely low irrespective of the factors such as the natural environment (geological setting, climate and topography), source of water composition, activities in the nearby water source. No toxic heavy metals (lead, mercury, cadmium and arsenic) was seen, bottle drinking water is safe concerning the heavy metals.

The number and type of parameters reported on the labels of bottled water showed a lack of homogeneity. Basic parameters (major ions) were usually indicated, whereas, for some brands generalized parameters were observed. In general, the concentration of species measured in this study was comparable to or slightly lower than values reported on the labels

5.2 Recommendations

From the observations of this study the following are the recommendations:-

- (i) Sources of bottled drinking water should be routinely monitored by both manufacturers and enforcers to ascertain its suitability for drinking and other purposes.
- (ii) The manufacturers should consider balancing the lost essential elements during water processing while the consumers of water should not depend on water as a source of these elements.
- (iii) The manufacturers should consider labeling all four major essential elements as directed by TBS to display the amount contained in the bottles.