SAFETY AND QUALITY OF STREAM AND BOREHOLE WATER USED BY SELECTED COMMUNITIES IN LUSHOTO DISTRICT, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

EXTENDED ABSTRACT

This study was conducted to assess the quality and safety of borehole and stream water used in Sunga and Mbaru wards in Lushoto district, Tanzania. Water samples from streams were collected in duplicate from three locations based on land use. These included forest areas, populated areas with agricultural activities and less populated areas with agricultural activities. Borehole's water was collected in duplicate from three boreholes in each ward. Samples from the two sources of water were analysed in triplicate using standard methods for chemical parameters (pH, Total hardness, ammonia, nitrate, phosphate, lead, arsenic and DDT) and microbiological parameters (E. coli and Salmonella). Nested design was applied and data obtained was analyzed by R-Software. Means were separated by using Tukey's honest at p<0.05. Results obtained were compared with TZS and WHO water guideline. Significant differences (p<0.05) in chemical parameters were observed in all locations within the streams except for arsenic. With the exception of phosphate in both streams all water samples tested met the TZS (2016) and WHO (2011). The phosphate levels were significantly higher (p<0.05) in populated areas in both streams but also in less populated areas in Daa stream than other study areas. All chemical parameters tested in borehole's water met the requirements for both TZS and WHO water guideline except pH and ammonia which exceeded the WHO (2011) water guideline. For microbiological parameters, significant differences (p<0.05) in E. coli and Salmonella were observed between the three locations of the streams. Furthermore significant differences (p<0.05) in E. coli contamination was also observed at boreholes water located at Madukani while the rest of boreholes were free from Salmonella and E. coli contamination. Good agricultural and hygienic practices should be applied so as to avoid contamination of water sources. Water from boreholes and streams should be treated before consumption to prevent water borne diseases.

DECLARATION

I, Hadija Athumani, do hereby declare to the Senate of Sokoine University of Agriculture						
that this dissertation is my own original work done within t	he period of registration and					
that it has neither been submitted nor being concurrent	tly submitted in any other					
institution.						
Hadija Athumani,	Date					
(MSc. Candidate)						
The above declaration is confirmed;						
Dr. L. Chove	Date					
(Supervisor)						

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DEDICATION

This work is dedicated to my beloved mother and my late father Mr. Twahiru Athuman who passed away during my data collection, may his soul continue to rest in peace.

I also dedicate this study to my beloved kids Anna, Arnold and Aaron, My beloved sisters for their love, encouragement and support.

TABLE OF CONTENTS

EXTEN	NDED ABSTRACTii
DECLA	ARATIONiii
COPYF	RIGHTiv
ACKNO	OWLEDGEMENTSv
DEDIC	CATIONvi
TABLE	E OF CONTENTSvii
LISTS	OF TABLESxi
LIST O	OF FIGURESxii
LIST O	OF MANUSCRIPTSxiii
LIST O	F ABBREVIATIONS AND SYMBOLSxiv
СНАРТ	ΓER ONE
1.0 In	ntroduction1
1.1 B	ackground Information
1.2 Pr	roblem Statement and Study Justification
1.3 O	Objectives of the Study
1.	.3.1 General objective
1.	.3.2 Specific objectives
1.4 R	eferences
СНАРТ	ΓER TWO
Paper C	One
Chemic	cal Quality of Stream and Borehole Water Used By Selected Communities
in Lush	oto District, Tanzania8

2.1	Abstrac	ct	8		
2.2	Introdu	ction	9		
2.3	Materia	al and Metho	ods11		
	2.3.1	Study area	a11		
	2.3.2	Materials	and reagents		
	2.3.3	Study des	ign		
		2.3.3.1	Sampling plan and data collection		
		2.3.3.2	Sample storage and preservation		
	2.3.4	Chemical	analysis of stream and borehole water		
		2.3.4.1	pH14		
		2.3.4.2	Total hardness		
		2.3.4.3	Ammonia		
		2.3.4.4	Heavy metal (Pb and As)		
		2.3.4.4	Dichlorodiphenyltrichloroethane (DDT)		
		2.3.4.4	Chromatographic condition used		
		2.3.4.5	Nitrate		
		2.3.4.6	Phosphate		
		2.3.4.7	Statistical data analysis		
2.4	Results and Discussion				
	2.4.1	Chemical	properties of the stream water in the two wards		
		2.4.1.1	The effect of location nested within and among the streams 18		
			2.4.1.1.1 pH		
			2.4.1.1.2 Total Hardness		
			2.4.1.1.3 Nitrate		
			2.4.1.1.4 Phosphate		
			2.4.1.1.5 Ammonia		

		2.4.1.1.6	Lead	. 24
		2.4.1.1.7	Arsenic	. 25
		2.4.1.1.8	DDT	. 26
	2.4.1.2	Chemical	parameters of the boreholes water found in	
		two ward	S	. 26
		2.4.1.2.1	pH	. 28
		2.4.1.2.2	Hardness	. 28
		2.4.1.2.3	Ammonia	. 29
		2.4.1.2.4	Nitrate	. 30
		2.4.1.2.5	Phosphate	. 31
		2.4.1.2.6	Arsenic	. 31
		2.4.1.2.7	Lead	. 32
		2.4.1.2.8	DDT	. 32
2.5	Conclusion			. 33
2.6	Recommendations	•••••		. 33
2.7	References			. 35
CHA	APTER THREE			. 43
Pape	r Two			. 43
Micr	obiological Quality	of Stream a	nd Borehole Water in	
Lush	oto District, Tanzan	ia		. 43
3.1	Abstract			. 43
3.2	Introduction			. 45
3.3	Material and Meth	ods		. 46
	3.3.1 Study area	a		. 46
	3.3.2 Materials			. 47

	3.3.3	Study desi	gn	47
		3.3.3.1	Sampling plan and data collection	48
	3.3.4	Microbiol	ogical analyses	48
		3.3.4.1	Detection and Enumeration of Escherichia coli	48
		3.3.4.2	Detection of Salmonella species	49
	3.3.5	Statistical	analysis	49
3.4	Results and Discussion			
	3.4.1	Microbiol	ogical parameters	49
		3.4.1.1	Location nested within the Streams and between	
			the streams	49
		3.4.1.2	The effect of location on stream contamination by E. coli	50
		3.4.1.3	The effects of location on stream contamination by	
			Salmonella	53
		3.4.1.4	Prevalence of E. coli and Salmonella among the	
			borehole water found in two wards	54
3.5	Conclu	sion		57
3.6	Recom	mendation		58
3.7	Referen	nces		60
CHA	APTER F	OUR		69
4.0	Overall	Conclusion	s and Recommendations	69
4.1	Conclu	sions		69
4.2	Recom	mendations		69
APP	ENDICE	ES		71

LISTS OF TABLES

Table 2.1:	Chemical properties of water from three locations nested
	within the streams
Table 2.2:	Chemical properties of water from three boreholes located at
	Sunga and Mbaru wards in Lushoto districts27
Table 3.2:	The mean value for <i>E. col</i> i and Salmonella in borehole water
	found in two wards55

LIST OF FIGURES

Figure 2.1: Map of Lushoto district showing location of the study area	12
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LIST OF MANUSCRIPTS

- Paper 1: Chemical quality of stream and borehole water used by selected communities in Lushoto district, Tanzania
- **Paper 2**: Microbial quality of stream and borehole water used by selected communities in Lushoto district, Tanzania

LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA Analysis of Variance

AOAC Association of Official Analytical Chemists

APHA American Public Health Association

As Arsenic

CaCO₃ Calcium carbonate

DDT Dichlorodiphenyltrichloroethane

EPA Environmental protection agency

GC- MS/MS Gas Chromatography-Triple Quadrupole Mass Spectrometer

ICP – MS Inductively Coupled Plasma – Mass Spectrometry

ISO International Organization for Standardization

mg milligram

mL milliliter

NGOs Non-governmental organizations

Pb Lead

pH Hydrogen potential

SUA Sokoine University of Agriculture

TBS Tanzania Bureau of Standards

URT United Republic of Tanzania

UV Ultraviolet

WHO World Health Organization

CHAPTER ONE

1.0 Introduction

1.1 Background Information

Water is a very basic natural resource for human life, and for socio-economic development. However, access to quality and safe water for community consumption is a global challenge (Addisie, 2012). This is due to uneven distribution of water over space, time, quantity and quality (URT, 2002).

For the socio-economic development to be achieved, the health of the community and their wellbeing requires the use of quality and safe water (WHO, 2011). Limited access to quality and safe water for community consumption makes them more vulnerable to waterborne diseases (WHO, 2011) and may eventually cause death. Therefore, providing quality and safe water to poor communities is a necessity, since it is a key determinant of the health of the community (Bradford *et al.*, 2016).

Various sources of contamination have been identified in drinking water including pathogenic bacteria such as *Salmonella*, *Escherichia coli* (Okoro *et al.*, 2017). Others are chemicals which can occur due to industrial or municipal discharges such as arsenic, lead, nitrate, and pesticides (Calderon, 2000). Exposure to these chemical contaminants can cause cancers, adverse reproductive outcomes and neurological diseases.

Water quality is an important attribute as it plays a vital role in the development of communities. Studies on water quality conducted in Tanzania has focused on underground water particularly boreholes. Most of these studies revealed that the water was of poor microbiological quality. These include a study by Kihupi *et al.* (2016) who

found that 63.6% of borehole water analysed were contaminated with fecal coliforms hence unfit for human use. Another study reported by Shayo *et al.* (2007) indicated high microbial loads specifically total viable count and fecal coliform. A study conducted in Dar Es Salaam by Basamba *et al.* (2013) indicated high fecal coliforms contamination. Other studies with similar conclusions, include a study by Kiangi (2014) and Chove *et al.* (2017).

Globally, 1.6 million people die annually due to diarrhea because of contaminated drinking water (Rufener *et al.*, 2010). Most of the rural communities are still using untreated water from streams, rivers and wells for various domestic uses (Onda *et al.*, 2013). The Tanzania rural communities, Lushoto district not being an exception, use water from unprotected streams, and rivers for cooking and drinking (Wagner and Lugazo, 2011). This may results into sickness and may cause death (URT, 2002). Therefore, by undertaking this study, such problems can be addressed and possible measures proposed.

1.2 Problem Statement and Study Justification

Lushoto District is located in Tanga region with an estimated population of 492,441 (URT, 2013). The district has many rivers and streams, which originate from the upland of Usambara Mountains (Mascarenhas, 2000). Due to the increase in population, high demand on access to water for both domestic and social economic activities is becoming vital.

The district is a major producer of fruits and vegetables for rural and urban markets. However, due to unreliable rainfall, water from these streams has been used for irrigation (Sokoni and Shechambo, 2005). During irrigation or rain, chemicals that are used as

fertilizers are further taken to the rivers and streams resulting into water pollution, and consequently affect water users down the stream.

Access to quality and safe water has become a challenge that make the community vulnerable to waterborne diseases like typhoid, dysentery and cholera (WHO, 2011). The majority of people living in Lushoto and Korogwe districts lack access to safe water for domestic consumption (Wagner and Lugazo, 2011). A study by Wagner and Lugazo, (2011) reported that about 52% of community members interviewed in different wards in Lushoto district have experienced water borne diseases to children and adults such as typhoid 22%, and diarrhea 1%. In order to address these problems the government and the non-governmental organizations (NGOs) introduced piped water in the district (Wagner and Lugazo, 2011). However, the supplied piped water is inadequate to meet the needs of the available population. In addition, the quality and safety of piped water is not regularly monitored which hinder their performance.

Available research related to water in Lushoto district has focused on local community participation in water management, local irrigation system used and the decline of water in the district (Mdendemi, 2013). Studies conducted to determine the quality and safety of stream and borehole water consumed by the local community in the district is limited. Hence, there is a need to establish the quality and safety of water used by the community so as to make recommendations for improving water quality and safety.

1.3 Objectives of the Study

1.3.1 General objective

Assessment of safety and quality of stream and borehole water used by selected communities in Lushoto District

1.3.2 Specific objectives

The specific objectives are to

- i. Determine chemical quality of stream and borehole water used in the selected villages of Lushoto district.
- ii. Assess the microbiological quality of stream and water used in the selected villages of Lushoto district

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CHAPTER TWO

Paper One

Chemical Quality of Stream and Borehole Water Used By Selected Communities in Lushoto District, Tanzania

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2.1 Abstract

A study was conducted to assess chemical quality of water in two streams and three boreholes from Sunga and Mbaru wards in Lushoto district, Tanzania. Water samples from streams were collected in duplicate from three locations including forest areas, populated area with agricultural activities and also less populated area with agricultural activities. Borehole's water was collected in duplicate from three boreholes found in each ward and were analyzed in triplicate for each parameter by using standard methods. Nested design was applied and data obtained was analyzed by R-Software for ANOVA. Means were separated by using Tukey's honest at p<0.05. Results obtained were compared with TZS and WHO water guideline. Significant differences (p<0.05) in all chemical parameters were observed in locations within the streams except arsenic. DDT was not detected in any water samples from the two sources. This implies that farmers in

Lushoto have followed the regulation and hence consumer protection is guaranteed. With exception of phosphate in stream water at populated area with agricultural activities in both stream and less populated area with agricultural activities in Daa stream, all water samples tested met the TZS (2016) and WHO (2011). All the parameters tested in borehole water met the requirements for both standards except pH and ammonia which exceeded the WHO water guideline. This implies that stream water is considered unsafe for use since it exceeded the maximum phosphate limit by TZS (2016). Therefore educating communities on the best practices to keep the water sources safe is very crucial especially in stream water which is close to agricultural activities in the district.

2.2 Introduction

Water is abundant in nature and is an important part of the earthly environment, covering about 75% of the earth surface. It occurs as surface water in lakes, streams, rivers, ponds, and as ground water which is obtained as spring, well, and borehole water (Chandra *et al.*, 2012).

Drinking water is defined as potable water intended for human consumption (TZS, 2016). Potable water shall be free from chemical substances that are hazardous and injurious (TZS, 2016). Surface water pollution remains a major problem worldwide, caused by both natural processes and anthropogenic activities (Noori *et al.*, 2010). Assessment of surface water quality in drinking water sources is vital as they can be one of the main pathways for the spreading of toxic chemicals and pathogenic microorganisms (Ouyang, 2005). The quality of surface water (stream) can be affected by point source and non-point sources of pollution (Nnane *et al.*, 2011). Point source pollution occurs from a particular identifiable source such as effluents from industries and wastewater treatment plants whereas non-point sources are runoff associated with a certain land use pattern such as

sewage overflows, agriculture (e.g., fertilizers, pesticides, animal manure), or forestry land uses (Hill, 2010). Surface water has been reported to be poor in quality, since there are prone to contamination (Okeola *et al.*, 2010). Agricultural activities are the source of chemical contaminants in water sources since they involve the use of fertilizers, herbicides and pesticides which produce toxic substances that are transported as effluents into water sources (Obi *et al.*, 2007). Other sources of water pollution include industries and human activities. It has been reported that some of chemical contaminants were of health concern these include nitrate, which rises due to excess fertilizers and can cause methaemoglobinaemia (WHO, 2011). Heavy metals are found naturally on earth and become concentrated as a result of human activities. Common sources are from mining and industries. Lead, for example can cause adverse neurological effects whereas arsenic can cause cancer and skin lesion.

In general, inadequate supply of safe and quality water is still a challenge in developing countries. The Joint Monitoring Programme (JMP) for Water Supply and Sanitation, implemented by the World Health Organisation (WHO) and UNICEF, reported that 783 million people in the world (11% of the total population) have no access to safe water, 84% of whom live in the rural areas. In Tanzania, the most common water source used in urban area is pipe water, although groundwater is also used as a supplemental source to meet the demand. About 31.7 % of the populations living in rural areas of Tanzania rely on water from ecosystem sources (i.e. springs, streams, rivers, ponds and lakes) which are more vulnerable to all kinds of contaminants (Noel, 2011). Despite the fact that Lushoto district depends on stream water as well as borehole water for cooking and drinking and there is a lot of agricultural activities along the stream. However there is limited information on the chemical quality of water used by community in the district. Hence,

there is a need to establish the quality and safety of water used so as to make recommendations for improving the safety and quality of water.

2.3 Material and Methods

2.3.1 Study area

The study was carried out at Mbaru and Sunga wards in Lushoto district, Tanga. The district is situated in the northern part of Tanga Region. It lies between latitude 4°25 and 4°55'S, and longitude 30°10 and 38°35E. It is one of the eight districts of Tanga Region, with a total area of 4092 Km² (URT, 2013). The main sources of water for the district are springs, streams and boreholes, where streams flow down the slopes of Usambara Mountains (URT, 2013). Previously, these streams were flowing throughout the year but recently the volume of water tends to decrease during the dry season from July to October (personal communication with local leaders). Changes in water quantity were attributed to replacement of natural forests by pine plantations as well as deforestation. Lushoto district has been selected as a study area due to the fact that communities depend on streams as well as boreholes as sources of water for drinking and other domestic purposes.

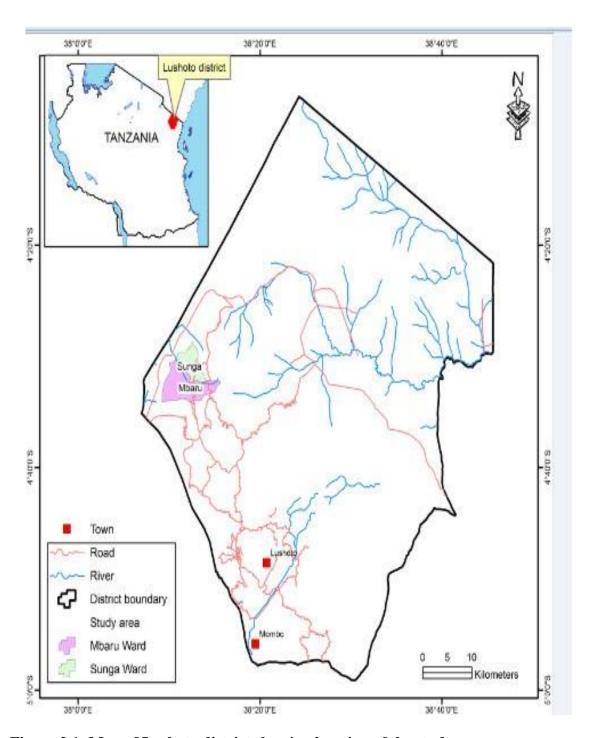


Figure 2.1: Map of Lushoto district showing location of the study area

2.3.2 Materials and reagents

Materials used for this study were water samples from the streams and boreholes. Others include the chemicals and reagents which were analytical grade, double distilled water used for rinsing and dilution/preparation of chemical solution. Cool box and sampling containers were also used.

Equipment used included ICP-MS (Model 7900- Agilent technologies, made in Germany), GC- MS/MS (model 7010 - Agilent technologies, made in Germany), Spectrophotometer (Model UV 2601 - Rayleigh, made in China), Colorimeter (Model DR890, Hach from U.S.A), Centrifuge (Model 300R-Hettich, made in German), Vortex -Talboys (Troemner LLC, made in U.S.A) and pH meter (model Orion 4 star plus, Thermo scientific, from U.S.A).

2.3.3 Study design

Cross sectional design was used in this study. Samples for chemical parameters (pH, total hardness, nitrate, ammonium, lead, arsenic and DDT) were drawn from three points for each of the two streams: forest, populated with agricultural activities, less populated and agricultural activities. The same design was also applied to boreholes water.

2.3.3.1 Sampling plan and data collection

Purposive sampling plan was used to collect samples from selected boreholes and streams found in two wards in Lushoto district. Sampling was carried out during the dry season from November to December 2018. Samples were obtained from two streams; Shagayu in Mbaru ward and Daa in Sunga ward. Borehole water was also obtained from the same wards. Water samples from the streams and boreholes were collected in the morning in a well labeled 1 Litre plastic bottles. Stream water was collected in duplicate at three points

(6 samples from each stream analyzed in triplicate to make a total of 18 samples for analysis per stream and hence a total of 36 data for each parameter). Water samples from the borehole were also collected in duplicate from the three boreholes found in each ward and analyzed in triplicate (6 samples in triplicate, making a total of 18 samples for analysis of each parameter). A tap from the borehole was allowed to run to waste for 3 minutes followed by rinsing of the 1 Litre plastic bottles with borehole water twice, prior to sample collection. Plastic bottles used to collect the samples were thoroughly washed and rinsed with distilled water prior to water collection. Analysis of pH was carried out at the water source. Water samples were then stored in an insulated ice box maintained at 4°C and transported to the Tanzania Bureau of Standards (TBS) laboratory for determination of heavy metal (lead and arsenic) and pesticide (dichloro-diphenyl-trichloroethane DDT). Other samples were transported to Tanga water laboratory for determination of other chemical parameters (total hardness, phosphate, nitrate and ammonia).

2.3.3.2 Sample storage and preservation

Upon arrival at the designated laboratories, the collected water samples from both sources (boreholes and streams) were stored in a refrigerator maintained at 4° C before analysis. However, samples for heavy metals analysis were first acidified with concentrated nitric acid (HNO₃) to lower the pH to < 2 (Aremu *et al.*, 2011). They were then kept in a refrigerator.

2.3.4 Chemical analysis of stream and borehole water

2.3.4.1 pH

The pH of the water samples was measured according to ISO 10523:2008. Results were reported in two decimal points.

2.3.4.2 Total hardness

Total hardness of water samples was determined by using 0.01 N of ethylene di amine tetra acetic acid (EDTA) titrimetric method as described in the standard methods for the examination of water and waste water according to the American Public Health Association (APHA, 2012). Results were reported as mg CaCO3/L.

2.3.4.3 Ammonia

The amount of ammonia in water from the two sources was determined by using calorimeter (Model DR890 Hach, from U.S.A). This was followed by analysis according to Nessler method 8038 which was adapted from Standard Methods for the Examination of Water and Wastewater (APHA). Results were reported in mg/L.

2.3.4.4 Heavy metal (Pb and As)

Analysis of heavy metal was done according to standard operating procedure (SOP) no FCL/SOPTM/13-03 which followed EPA Method 6020 and Agilent 7900 ICP-MS Manual. Blank and standard calibrations were used where by four levels of mixed standards solution of arsenic (As) and lead (Pb) (10 ppb, 25 ppb,50 ppb and 75 ppb) were used to prepare calibration curve which was used to quantify concentration of lead and arsenic in water samples. Quality control of 0.5ppb mixed standards and blank sample (distilled water) were also run alongside the water samples. Results were reported in mg/L.

2.3.4.4 Dichlorodiphenyltrichloroethane (DDT)

Determination of Dichlorodiphenyltrichloroethane (DDT) in water was carried out by using standards operating procedure (SOP) no FCL/SOP-TM/14 modified from AOAC Official Method 2007.01 by using Gas chromatography Tandem Mass Spectrometer

(GC MSMS, model 7010 Agilent technologies, German). Extraction of DDT was done by weighing 10 g of water sample into 50 mL of teflon tube, followed by addition of 10ml of acetonitrile to the Teflon tube, mixed and allowed to vortex for a minute, then 1g of sodium chloride, 4 g of anhydrous magnesium sulfite, 1 g of trisodium citrate diyhdrate and 0.5 g of Disodium hydrogen citrate sesquhydrate were added to the mixture shaken and allowed to vortex again for a minute and centrifuged at 5000rpm for 5 minutes. About 7.5 ml of supernatant was pipetted into 15 ml Teflon tube. Thereafter 750 mg of MgSO4 and 150 mg PSA were added and mixed by vortexing for 1 minute. The mixture was then centrifuged at 5000 rpm for 5 minutes. About 5 ml of extracted solution was taken into a graduated centrifuge tube and evaporated to nearly dryness under nitrogen (the temperature of water samples were below 42 °C) then reconstituted to 1 ml with toluene, homogenize with vortex mixer for a 5 sec. Triphenyl phosphate (TPP) was used as internal standards. The concentrated extract was then transferred to vials for GC injection. Prepared four levels of standard solution were run to prepare calibration curve and quantify the concentration of total DDT. Also 50ppb of spiked sample with triphenyl phosphate (TPP), and blank itself (distilled water) were also run alongside with water samples. Quantification and results calculation was done by mass hunter software using the following formula.

Concentration of each analyte ($\mu g/L$) = Concentration from curve X dilution factor Where by Concentration from curve = Peak Area of the analyte / Peak area of internal standard.

2.3.4.4 Chromatographic condition used

GC column -15 mmx0.25 mm x0.25 mm HP-5MS part number 19091S-433 (Agilent, U.S.A).

Inlet, Carrier gas: He (Flow rate 1.5 mL/ min Injection volume - 1 µl)

Inlet temperature − 280 °C,

Inlet mode-spilt-less, Purge flow to spilt vent: 30 mL/min at 0.75 min,

Gas saver on (20 mL/ min at 2.0min),

Inlet liner –split-less, single taper.

2.3.4.5 Nitrate

The amount of nitrate in water was determined by using 4500-NO3–B where by Ultraviolet Spectrophotometric(Model UV 2601 – Rayleigh, made in China) was used to measure the absorbance of the water samples as described by American Public Health Association (APHA,2012). Results were expressed in mg/L

2.3.4.6 Phosphate

The amount of phosphate in water was determined by ascorbic acid method as described in (APHA, 2012) and absorbance was measured at 880 nm. Results were reported in mg/ L.

2.3.4.7 Statistical data analysis

Nested design was applied using the following model

$$Y_{ijk} = \mu + \beta_j + \alpha_{(j)i} + \varepsilon_{ijk}$$
 and $Y_{ijk} = \mu + \lambda_k + \rho_{(k)\chi} + \varepsilon_{ijk}$

Where by:

 Y_{ijk} = Dependant variable, μ =General mean, β_j = 1, 2, (stream),

 $\alpha_{(j)i} = 1, 2,3$ (effect of location nested within stream), $\lambda_{k=1,2(ward)}$,

 $\rho_{(k)}$ (effect of borehole nested within the ward), and ε_{ijk} Random error

Data was analyzed using R- statistical package software. Nested design was applied on the stream and boreholes water to determine the effect of location nested within a stream and effect of boreholes in the wards. Analysis of Variance (ANOVA) was carried out to determine the significant difference between the location within the stream and boreholes. Means were separated using Tukey's Honest at p<0.05.

2.4 Results and Discussion

2.4.1 Chemical properties of the stream water in the two wards

2.4.1.1 The effect of location nested within and among the streams

The chemical properties of streams studied are presented in Table 2.1. These results summarizes the mean values and standard deviation for pH, total hardness (T.H), Nitrate (NO₃), PO4³⁻ (Phosphate), NH₃ (Ammonia), Arsenic (As), Lead (Pb), and Dichloro-diphenyl-trichloroethane (DDT).

Table 2.1: Chemical properties of water from three locations nested within the streams

Stream			Parameter						
A	Position	pН	Hardness (mg/L)	NO ₃ (mg/L)	PO ₄ (mg/L)	NH ₃ (mg/L)	Pb (mg/L)	As (mg/L)	DDT(µg/
									L)
	1A0	6.92 ± 0.02^{a}	23.88 ± 0.33^{a}	1.73 ± 0.01^{a}	0.90 ± 0.01^{b}	0.08 ± 0.01^{a}	0.001 ± 0.0^{a}	0.0001 ± 0.0	ND
	2A1	7.23 ± 0.02^{b}	34.28 ± 0.50^{b}	5.95±0.19 ^c	*2.50±0.09 ^e	0.07 ± 0.01^{a}	0.002 ± 0^{b}	$0.0001\pm0.$	ND
								0^{a}	
	3A2	7.34 ± 0.01^{b}	38.20 ± 0.23^{c}	6.81 ± 0.12^{d}	*3.90±0.06 ^f	0.06 ± 0.02^{a}	0.003 ± 0^{c}	0.0001 ± 0^{a}	ND
MEAN		7.15 ± 0.18	32.12 ± 6.23	4.83±2.29	2.45 ± 1.29	0.06 ± 0.02	0.002 ± 0.0	$0.0001\pm0.$	ND
							01	0	
В	1B0	6.98 ± 0.05^a	43.40 ± 0.40^{d}	5.00 ± 0.11^{b}	0.58 ± 0.03^{a}	$0.02\pm0.01^{\rm \ b}$	0.001 ± 0^a	0.0001 ± 0^{a}	ND
	2B1	6.92 ± 0.04^a	42.50 ± 0.50^{d}	$19.80 \pm 0.28^{\mathrm{f}}$	*2.36±0.06 ^d	0.07 ± 0.005^{a}	0.001 ± 0^a	0.0002 ± 0^a	ND
	3B2	6.97 ± 0.07^a	$64.43\pm0.45^{\rm e}$	14.24±0.28 ^e	2.00±0.05°	0.03 ± 0.01^{b}	0.001 ± 0^{a}	0.0001±0°a	ND
MEAN		6.96 ± 0.06	51.07±9.76	13.01±6.29	1.63 ± 0.78	0.09 ± 0.06	0.001 ± 00	0.0001 ± 0	ND
TZS 789		5.5-9.5	600	45	2.2	0.5	0.01	0.01	1
WHO 2011		6.5-8.5	500	50	NR	0.2	0.01	0.01	1

Values in the same column having the same superscript letters are not significantly different (p > 0.05) (Tukey's Honest)

NR- Not a requirement.

A-Daa stream; B-Shagayu stream

1A0-Forest for stream A, 2A1- Kwamamkoa (populated with agricultural activities), 3A2- Komboheo (less populated with agricultural activities),

1B0-Forest for stream B, 2B1- Ludende (populated with agricultural activities), 3B2- Kumbamtoni (less populated with agricultural activities)

ND- Not Detected,

^{*} Failed to meet the standard requirement

2.4.1.1.1 pH

The mean pH of water in the two streams ranged between 6.92±0.04 -7.34±0.01. No significant differences in pH were found between the water samples in Shagayu stream. The pH of the water from forest area in Daa stream was low and significantly different (p<0.05) from the two areas with agricultural activities. Change in pH within the stream might be attributed to the fact that most of the open water bodies are exposed to various pollutants that can influence the variation of pH (Napacho and Manyele, 2010). The use of alkaline detergent for washing clothes and motorcycles were observed in the nearby streams and discharge of alkaline waste water from the household which can result in increase in pH. This observation is also supported by Napacho and Manyele (2010) whose study reported the pH ranged from 7.8-8.0 in stream and suggested that high pH value obtained could be attributed by different activities done near the stream such as washing clothes and cars. Related observation was also reported by Chang (2008) who observed that increases in pH in stream water were associated with increasing the use of alkaline detergents and alkaline material from wastewater from the household.

In this study it was revealed that pH recorded at the forest in both streams was slightly acidic. This might be associated with decomposition of pine tree which may add acidity to the soil and influence the acidity of nearby stream. This finding corroborate to the study reported by Tremblay *et al.* (2009) who found that decrease of pH in water stream in Montmorency forest in Canada was due to release of organic acid from decomposition of trimmed branches of tree. Furthermore, the mean pH values for the two streams showed a slight variation (Table 2.1) which might be attributed by soil type and land use activities along the respective streams. This observation had been reported by Njue *et al.* (2016). It was found that soil and land use activities affects the proportion of major ions in water bodies. However, since all the pH values recorded met the standards as recommended by

both TZS (2016) and WHO (2011), the water in the area is considered safe with respect to pH.

2.4.1.1.2 Total Hardness

The study results for total hardness in three locations within two streams ranged from 23.88±0.33 mg/L to 64.43±0.45 mg/L. Although no significant differences observed in total hardness between the forest area and Ludende in Shagayu stream, these differences were obvious in the rest of the locations (Table 2.1). Non-significant differences observed in two locations might be influenced by similar geology of particular locations. A previous study conducted by Seiyaboh and Izah (2017) assessed the impact of a anthropogenic activities in stream water found total hardness ranged from 38.3 -50 mg/L. Likewise Yisa and Jimoh (2010) reported total hardness ranged from 33-60 mg/L.

Wannamethee *et al.* (2011) reported that there was no serious health effect due to consumption of hard water but in a very rare case it could be associated with human disease like cardiovascular and cerebrovascular particularly to elderly people. Furthermore, the hardness of water is not considered as a pollution parameter but an indication of low salinity due to the presence of calcium and magnesium ions expressed as CaCO₃ (temporary hardness). Moreover, since all water samples drawn met the TZS (2016) and WHO (2011) maximum allowable limit, the water is considered safe for consumption as far as total hardness is concerned.

2.4.1.1.3 Nitrate

The mean nitrate in the two streams ranged between 1.73±0.01 mg/L to 19.80±0.28 mg/L. There were significant differences (p<0.05) in nitrate within locations in the two streams. Low level of nitrate was recorded at the forest; this might be attributed to the fact at that

particular location, there was no agricultural activity or human settlement which could influence the rise of nitrate. A study reported by Jacobs *et al.* (2017) indicated mean nitrate concentration ranged from 0.30 ± 0.08 mg/L to 0.55 ± 0.15 mg/L in Montane stream water in Kenya which was near the natural forest.

Increased level of nitrate was observed in both populated and less populated areas with agricultural activities in both streams compared to the forest. This may probably due to application of fertilizers in farms, discharge of wastes that ultimately end up in the stream. The same finding was previously reported (Jacobs *et al.*, 2017; Ngoye and Machiwa, 2004). These authors found that increased level of nitrate in water was due to application of fertilizer in agricultural area which ended up in the stream. Although nitrate is considered to be of less environmental problem in high concentration however, (above 40 mg/L) it may lead to a disease called "Methaemoglobinemia" or "blue baby syndrome" in children (Sarda and Sadgir, 2015).

Shagayu stream had a relatively higher level of nitrate than Daa (Table 2.1). Higher levels of nitrate might be due to cultivation of mixed crops along the stream such as potatoes, carrots and cabbages which require greater input of fertilizers which contribute to nitrate leaching from the soil to the stream. The major source of nitrate is from domestic sewage, animal waste, agricultural waste and runoff from the settlement (Christensen *et al.*, 2012). Since all water sampled from the three locations within the streams met the recommended limit by TZS (2016) and WHO (2011), it is considered safe for use, as far as nitrate is concerned.

2.4.1.1.4 Phosphate

Mean phosphate value within the three locations in Daa and Shagayu streams ranged from 0.58±0.03 mg/L to 3.90±0.06 mg/L. Significant differences (p<0.05) in phosphate was

observed in all locations within both streams as shown in Table 2.1. The highest level of phosphate was observed in Daa stream at Komboheo (agricultural with less population) while the lowest level observed at the forest in Shagayu stream. With the exception of forest in both streams and Kumbamtoi in Shagayu stream, other locations observed had higher levels of phosphate than those recommended by the Tanzania standard. The higher level of phosphate recorded at Komboheo might be associated with its location. Komboheo is located down the stream where by all the detergents poured and flushed by people washing their clothes at Kwamamkoa (midstream) may have been washed and flowing downward and hence raise the phosphate level downstream. Studies conducted by Saria (2015) and Fadiran *et al.* (2008) found that, the increased level of phosphate in stream close to agricultural area may be caused by the application of fertilizers near the stream as well as detergents from the households.

It has been reported that higher concentration of phosphate in water can affect the digestive system of animal and human (Dawood *et al.*, 2014). Furthermore, the mean phosphate level recorded in two streams was shown (Table 2.1). Regarding to this, Daa stream was more polluted and did not comply with the recommended Tanzania standard while Shagayu stream met the requirement. However, Ludende exceeded the limit set by the Tanzania standard. WHO has not established the limit of phosphate in drinking water. It was therefore concluded that Daa stream is not safe for human use because the mean phosphate value is above the TZS recommended level which is 2.2 mg/L (TZS, 2016).

2.4.1.1.5 Ammonia

The mean value for ammonia in both streams at the three locations ranged from 0.02±0.01 mg/L to 0.08±0.01 mg/L (Table 2.1). Significant differences (p<0.05) in ammonia were observed in two locations found in Shagayu stream including forest area

and less populated area with agricultural activities. Low level of ammonia observed at the forest may probably due to the fact that there were neither agricultural activities nor human settlement which could discharge wastes to the water bodies. This finding is also supported by Ngoye and Machiwa (2004) and Huang *et al.* (2013). Moreover low concentration of ammonia observed at Ludende and Kwamamkoa (populated with agricultural activities) is contrary to the finding reported by Ngoye and Machiwa (2004) who found high level of ammonia (from 1.3 ± 0.7 mg/L to 2.6 ± 0.6 mg/L) in the area where stream water was adjacent to agricultural activities.

Results of ammonia at Kumbamtoi and Komboheo (less populated area with agricultural activities) was in agreement with the finding reported by Effendi *et al.* (2015) who found ammonia ranged from 0.0059 -0.0178 mg/L. In his observation he stated that low level of ammonia was due to low population and less application of inorganic fertilizer. It has been reported that there was no health-based guideline proposed due consumption of water contaminated with ammonia. However, it can compromise disinfection efficiency, resulting in nitrite which causes the failure of filters for the removal of manganese and cause taste and odour problems (WHO, 2003). The mean values for the two streams are indicated in Table 2.1.Therefore all samples from both streams met the requirement set by TZS (2016) and WHO (2011) and hence safe for human use with respect to ammonia.

2.4.1.1.6 Lead

Results for lead in two streams showed that all samples drawn from three locations had low level of lead which was below recommended limit by TZS (2016) and WHO (2011). Although the mean Lead levels were slightly higher in Kwamamkoa and Komboheo and statistically different (p<0.05) from all other locations, these results were within the acceptable limits by both the TZS (2016) and WHO (2011) water guideline. The slight

variation might be influenced by deposition of various wastes in the water body. Previous study reported by Nyairo *et al.* (2015) showed low level of lead with mean concentration of 0.009 mg/L in Amala streams of river Mara, Kenya which is adjacent to forest, agricultural area and human settlement.

Lead is mainly introduced into water bodies through different ways such as the disposal of batteries, agricultural runoff from fields that use sewage sludge as fertilizers, atmospheric deposition of exhaust from vehicles, and sewage discharge (Alsaffar *et al.*, 2016). High level of lead may lead to a wide range of effects, including neurodevelopmental effects, mortality due to cardiovascular diseases, impaired renal function, hypertension and impaired fertility (WHO, 2011). Regarding the quality of streams in terms of lead, it was found that Daa stream had a mean concentration of 0.002 ± 0.001 mg/L while Shagayu stream had 0.001 ± 0.00 mg/L. Therefore all the sampled water in both streams met the recommended standard by TZS (2016) and WHO (2011) and hence considered safe for human consumption with respect to lead.

2.4.1.1.7 Arsenic

The mean arsenic values in three locations of both streams are shown in Table 2.1. Results obtained from the three locations were very low, almost negligible. In addition there was no significant difference in arsenic levels (p>0.05) between the three locations in the two streams. Low level of arsenic might be attributed by non-application of arsenical pesticides in the study area. The mean arsenic values in the two streams had the same concentration as shown in Table 2.1. Arsenic level in water could be due to human activities such as application of arsenical pesticides in agricultural areas (Vowinkel *et al.*, 2001). High arsenic level in water can cause cancer in lungs, bladder and skin. Also skin lesions and peripheral vascular diseases have been reported in population consumed water

contaminated with arsenic (WHO, 2011). Moreover, since all the samples complied with the TZS (2016) and WHO (2011) then it may be concluded that water is safe for use with respect to arsenic

2.4.1.1.8 DDT

The water samples analysed from both streams were below detection limit for Dichloro-diphenyl-trichloroethane (DDT) which was 0.05 µg/L. This implies that all samples met the TZS (2016) and WHO (2011) recommendations. This might be attributed with the fact that the use of DDT in agriculture was banned in Tanzania since 1997 (URT, 2005). In human beings, the higher concentration of DDT leads to neuropsychological such as brain tumors (Leena *et al.*, 2012). A study by the same author found a concentration ranging from none to 0.49 µg/L of total DDT in the upper and downstream of river Ganga, India which was near agricultural area and human settlement. The source of the DDT was discharge of agrochemicals from flood plains and medical waste from hospital which was channeled direct to the river (Leena *et al.*, 2012). Therefore, since all water sampled met the requirement then water deemed safe for use with regard to DDT.

2.4.1.2 Chemical parameters of the boreholes water found in two wards

Water from the boreholes which were found within the two wards were tested and compared, and the summary of their results are presented in Table 2.2.

Table 2.2: Chemical properties of water from three boreholes located at Sunga and Mbaru wards in Lushoto districts

Ward	Code	Parameters							
		pН	Total Hardness	NH_3	$PO_4(mg/L)$	$NO_3(mg/L)$	As (mg/L)	Pb (mg/L)	$DDT(\mu g/L)$
			(mg/L)	(mg/L)					
Sunga	1 A	$^*6.37\pm0.10^{a}$	124.20±0.24°	0.08 ± 0.01^{b}	0.20 ± 0.02^{a}	7.83±0.11 ^a	0.0002 ± 0.00^{b}	0.005 ± 0.001^{d}	ND
	2A	6.73 ± 0.04^{b}	64.40 ± 0.39^{a}	0.02 ± 0.01^{a}	2.04 ± 0.02^{d}	13.00 ± 0.13^{b}	0.0001 ± 0.00^{a}	0.01 ± 0.001^{a}	ND
	3A	6.89 ± 0.01^{c}	67.40 ± 0.33^{b}	$^*0.32\pm0.01^{d}$	0.20 ± 0.01^{a}	0.60 ± 0.14^{c}	0.0001 ± 0.00^{a}	0.0001 ± 0.0^{c}	ND
Mean		6.67 ± 0.23	85.30 ± 28.3	0.14 ± 0.10	0.81 ± 0.80	8.79±3.10	0.0001 ± 0.00	0.006 ± 0.005	ND
Mbaru	1B	7.10 ± 0.01^{d}	65.60 ± 0.47^{a}	0.02 ± 0.01^{a}	0.6 ± 0.02^{b}	2.00 ± 0.02^{d}	0.0001 ± 0.00^{a}	0.01 ± 0.0^{a}	ND
	2B	7.10 ± 0.01^{d}	219.63±1.05 ^e	0.02 ± 0.00^{a}	0.20 ± 0.01^{a}	2.79 ± 0.07^{e}	0.0001 ± 0.00^{a}	$0.001\pm0.0^{\rm b}$	ND
	3B	$7.17\pm0.\ 10^{d}$	204.03 ± 1.36^{d}	0.18 ± 0.01^{c}	0.9 ± 0.05^{c}	$3.29\pm0.18^{\rm f}$	0.0001 ± 0.00^{a}	0.002 ± 0.0^{b}	ND
Mean		7.12±0.05	163.1±71.20	0.07±0.06	0.57±0.31	2.66±0.58	0.0001±0.0	0.004±0.004	ND
TZS 789)	5.5-9.5	600	0.5	2.2	45	0.01	0.01	1
WHO 20	011	6.5-8.5	500	0.2	NR	50	0.01	0.01	1

Values in the same column having the same superscript letters are not significantly different at p > 0.05 (Tukey's Honest)

¹A-Alufea, 2A-Madukani, 3A-Kwemashui,

¹B-Masereka, 2B-Ludende, 3B-Chambogo,

NR-Not a requirement,

ND-Not detected,

^{*} Failed to meet the WHO standards

2.4.1.2.1 pH

pH of the boreholes for the two wards ranged from 6.37±0.10 to 7.17±0.10. The pH of boreholes water from Sunga was neutral and was significantly different (p<0.05) from all other boreholes water from Mbaru which were below pH 7 (Table 2.2). The pH values recorded in this study are related to that of a previous study by Saana *et al.* (2016) which reported pH value ranging from 6.14–7.50. From the results obtained, the least pH value was observed at Alufea in Sunga ward while the maximum value was recorded at Chambogo (Table 2.2). The slight acidic pH observed might be attributed by soil type that permits dissolution of acidic materials from agriculture and which bring about slight acidity in the water (Oko *et al.*, 2014). In addition, discharge of acidic materials into the ground through agricultural and domestic activities might also attribute to acidic condition of the borehole water (Yusuph *et al.*, 2018). Neutral pH observed at three boreholes located at Mbaru ward showed no significant differences (p>0.05). These results are comparable with study reported by Christine *et al.* (2018) who recorded the neutral pH at the boreholes water located at Kakamega County in Kenya.

Long term exposure to pH beyond the permissible limit affects skin and the mucous membrane of cells (Nishtha *et al.*, 2012; Napacho and Manyele, 2010). Therefore with the exception of pH for sample drawn from Alufea which was below WHO limit, all water sampled met the TZS (2016) and WHO (2011). The mean pH of both wards (Table 2.2) met the requirement for both standards hence safe for human use with respect to pH.

2.4.1.2.2 Hardness

The total hardness in boreholes water ranged from 64.40±0.39 mg/L to 219.63±1.05 mg/L. There was a significant differences (p<0.05) in hardness for all boreholes in both wards. The greater variation observed in water hardness could probably be due to the

presence of minerals such as limestone in the soil of a respective area. Related study was also reported by Saana *et al.* (2016) who found the mean hardness of borehole water which ranged from 22 mg/L to 178.07 mg/L in six districts in the Northern region of Ghana.

According to Napacho and Manyeli (2010) hardness or softness in water varies from place to place due to nature of the geological properties of that particular area. Chigut *et al.* (2017) categorized water based on hardness as soft (75 mg/L), moderately hard (75–150 mg/L), hard (150–300 mg/L) and very hard (300 mg/L). Most of water sampled falls on moderate hard but very few falls on hard water including water sampled from Chambogo and Ludende. Hard water can cause formation of precipitates in piping and fittings which can cause water blockage and reduce the interior diameter of piping. However, long term consumption of extremely hard water might lead to an increased incidence of urolithiasis, prenatal mortality, and cardiovascular disorders (Shigut *et al.*, 2017; Wannamethee *et al.*, 2011). Therefore all water samples drawn from the two wards (Table 2.2) met the maximum allowable limit by TZS (2016) and WHO (2011). Therefore regarding total hardness borehole water recommended fit for human consumption.

2.4.1.2.3 Ammonia

Borehole water sampled ranged from the mean value of 0.02±0.0 mg/L to 0.32±0.01 mg/L. Significant differences (p<0.05) in ammonia were observed in boreholes located at Alufea, Kwemashui and Chambogo (Table 2.2). High level of ammonia was recorded at Kwemashui, this might be attributed to the fact that the borehole is located close to agricultural activities. A study by Adekola *et al.* (2015) found the mean level of ammonia in boreholes in Gassol, Nigeria to be 0.21 mg/L. The researchers explained that the high

level of ammonia could be due to agricultural activities from intensive rearing of farm animals. Therefore, with the exception of borehole located at kwemashui which was higher than the WHO acceptable limit, all the water sampled met the maximum recommended limit by TZS (2016) and WHO (2011). However the mean value of ammonia in both wards (Table 2.2) met the requirement for both standards. Therefore as per TZS (2016) all water samples are safe for use as far as ammonia is concerned.

2.4.1.2.4 Nitrate

The level of nitrate ranged from 0.60 ± 0.14 m/L to 13 ± 0.13 mg/L (Table 2.2). Significant differences in nitrate levels (p<0.05) were observed in all boreholes from the two wards. High level of nitrate revealed at Madukan might be caused by waste discharges from the household and agricultural activities taking place. This observation is also supported by other researchers (Oluma et al., 2010; Nkamare et al., 2012). They stated that although nitrate naturally occurred in groundwater, high concentration of nitrate could be associated with animal and human waste, decomposition of plant debris, nitrogen fertilizer, household solid waste or sewage discharge on land. Different studies reported the level of nitrate in borehole water including a study by Mpenyana et al. (2012) which reported concentration of nitrate ranged from 0.45-7.27 mg/L, Sanaa et al. (2016) reported nitrate ranged from 0 - 6.0 mg/L whereas Adekola et al. (2015) reported values ranging from 0.17 -32 mg/L. Excessive NO₃ in drinking water can cause a number of disorders including methemoglobinemia in infants, gastric cancer, goiter, birth malformations and hypertension as cited by Shigut et al. (2017). Variation in nitrate level observed in this study might be attributed by the fact that most of boreholes in Sunga ward were close to agricultural areas. Regardless of relatively high level of nitrate observed, all water sampled met the recommended limit by TZS (2016) and WHO (2011)

hence suggesting that concerning the nitrate level, borehole water is safe for human consumption.

2.4.1.2.5 Phosphate

The mean value for phosphate ranged from 0.20 ±0.01 mg/L to 2.04 ±0.02 mg/L. In Daa stream, significant differences in phosphate (p<0.05) were observed in borehole located at Madukani, Masereka and Chambogo. The highest value of phosphate was recorded at Madukani (Table 2.2) which might be attributed by domestic sewage, detergent since people wash their clothes and motocycles around the boreholes. In addition agricultural effluents with fertilizers could also contribute to the rises of phosphate in borehole water. This observation was also reported by other researchers (Murhekar, 2011; Oko *et al.*, 2014). A finding by Oko *et al.* (2014) reported the mean value of 1.14 mg/L in borehole water located in two wards in Wukari, Nigeria. Likewise the finding reported by Ukpong and Okon, (2013) found mean phosphate level in boreholes ranged from 0.01 mg/L -1.07 mg/L in Uruan local government area, Nigeria. High level of phosphate in water can affect the digestive system of both animal and human (Dawood *et al.*, 2014). The mean values of phosphate in two wards were shown (Table 2.2). Therefore all sampled water from both wards were below recommended level by TZS (2016) and concluded that borehole water are safe for use with regard to phosphate level.

2.4.1.2.6 Arsenic

For Arsenic parameter tested in borehole water, the mean value ranged from 0.0001 mg/L to 0.0002 mg/L. Significant differences (p<0.05) in arsenic level were observed in a borehole located at Alufea. This variation might be caused by soil type of a particular area since arsenic is natural occurring in rock and soil. This observation was also reported by Musa *et al.* (2008) who found that arsenic contamination in borehole was caused by

natural geological sources leaching into aquifers and disposal of arsenic containing materials. Previous study conducted by Musa *et al.* (2008) reported arsenic level in borehole water that ranged from 0.002 to 0.008 mg/ L. This is in agreement with the finding of this study. Long-term exposure to arsenic in drinking-water can cause skin lesions, skin cancer, lung and bladder cancer (Hilma *et al.*, 2016: WHO 2011). In addition, consumption of water contaminated with arsenic has been associated with cardiovascular disease in children an average of 7 years (WHO, 2011). Therefore all water samples complied with TZS (2016) and WHO (2011) and suggesting that with respect to arsenic borehole water is fit for human use.

2.4.1.2.7 Lead

The mean concentration of lead ranged from 0.0001 mg/L to 0.01 mg/L as shown in Table 2.2. Various studies conducted by Ukpong and Okon, (2013) and Chinedu *et al.* (2011), did not detect any lead at all. Exposure to lead in drinking water is associated with a wide range of effects, including various neurodevelopmental effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes (WHO, 2011). Therefore all water samples tested were within the recommended level by TZS (2016) and WHO (2011) water guideline. For that reason the borehole water is considered safe for use with regard to lead.

2.4.1.2.8 DDT

This pesticide was banned for use in Tanzania since 1997 (URT, 2005). All the samples tested for DDT were below detection limit which was 0.05µg/L. A study by Shukla *et al*. (2006) reported level of DDT ranged from 0.15-0.19 µg/L in underground water which is contrary to the finding of this study. In his observation he stated that concentration of DDT obtained was possibly due to transfer of organochlorine pesticides from agricultural

and health protection activities carried out near Hyderabad, India DDT has a possible long-term toxicity as it remains in the environment for a long time (WHO, 2011). Exposure to DDT in water may lead to human health including lung damage, cancer and injury of reproductive and nervous system (Mansour, 2004). Therefore based on these results, all samples analysed complied with the maximum limit recommended by TZS (2016) and WHO (2011). Borehole water is considered safe for human consumption due to non detectable levels of DDT in the analysed borehole water samples.

2.5 Conclusion

Access to quality and safe water is essential, regardless of the water source. In this study all tested samples from the two streams, fall within recommended level proposed by TZS and WHO except phosphate from shagayu (Ludende) and Daa stream (Kwamamkoa and Komboheo) which was found to be high in both areas with agricultural activities. Therefore, improper discharges of waste from the nearby streams and other points to the streams should be prohibited to keep water safe especially in populated areas with agricultural activities. For borehole water however, all chemical parameters tested were within the permissible limit by TZS and WHO except ammonia from Kwemashui and pH at Alufea in Sunga ward which were above WHO recommended levels. Despite the shortcoming, borehole water sampled in Lushoto district was found to be safe and of good quality based on the Tanzanian standard.

2.6 Recommendations

Based on the findings of this study the following are recommended:

 The same study to be conducted during the rainy season or both seasons for the purpose of comparing the findings whether there are similarities or differences between the two seasons.

- ii. To assess more parameters such as sulphate, fluoride, chloride, copper, conductivity, turbidity, biochemical oxygen demand, total suspended solid and pesticides as per TZS 789 for the purpose of getting a clear picture of the water sources used in terms of safety and quality. However information on the pesticides used in the study area is very crucial so that residue will be assessed based on the pesticide in use.
- iii. Government engagement through community health officers and other stakeholders in educating the community on the possible health risks associated with consumption of contaminated water from the two sources. The community should also learn about the best practices for keeping and maintaining the available boreholes and streams for the purpose of safeguarding the water sources
- iv. The community should also adhere to good agricultural practices and general hygienic practices so as to ensure that water sources are well protected from chemical contamination.

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CHAPTER THREE

Paper Two

Microbiological Quality of Stream and Borehole Water in Lushoto District, Tanzania

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3.1 Abstract

The aim of this study was to assess microbiological quality of water in two streams and three boreholes from Sunga and Mbaru wards in Lushoto district, Tanzania. Water samples from streams were collected in duplicate from three locations including forest areas, populated area with agricultural activities and also less populated area with agricultural activities. Borehole's water was also collected in duplicate from three boreholes in each ward and were analyzed in triplicate for each parameter by using standard methods. Nested design was applied and data obtained was analyzed by R-Software for ANOVA. Means were separated by using Tukey's honest at p<0.05. Results obtained were compared with TZS and WHO water guideline. Significant differences (p<0.05) in *E. coli* and *Salmonella* were observed in three locations of the streams (forest, agriculture with population and agriculture with less population). Agricultural area with population found to be highly contaminated with *E. coli* and

Salmonella, followed by agricultural with less population. With exception of *E. coli* in stream water at the forest in Daa stream other samples were not detected with *E. coli* and Salmonella. All water samples from the streams failed to meet the TZS and WHO water guideline. Furthermore, results for boreholes indicate significant deferences (p<0.05) in *E. coli* from the borehole water located at Madukani. Therefore with exception of *E. coli* in borehole from Madukani other samples met the requirements for both standards. Therefore regardless of the water sources, community members should treat water before consumption so as to avoid the risk of water borne diseases.

3.2 Introduction

Water is an essential component of life (Osunkiyesi, 2012). People need water in day to day activities such as cooking, washing, drinking and for industrial activities. Consuming safe drinking water is considered as a basic need for human right and for everyone (Peter-Ikechukwu *et al.*, 2015). Its accessibility to the society plays a crucial role not only in economic development and social welfare, but also an essential element in health (Eze and Eze, 2015).

About two thirds of drinking water consumed worldwide is derived from various sources such as lakes, stream, rivers and open wells. However, microbiologically, these sources can easily be contaminated by sewage discharges or fecal from domestic or wild animals (WHO, 2003). On global perspective, groundwater offer potable water to about 1.5 billion people daily. According to Parker (2006), more than one sixth of the world's populations lack access to safe drinking water. This is due to the fact that natural water are susceptible to contamination with microorganisms and other pollutants regardless of the source (Oludairo, 2015).

In sub-Saharan Africa, groundwater has proved to be the most reliable source for meeting rural water demand (Iyasele and Idiata, 2012). However, it is susceptible to microbial contamination, despite the fact that it is filtered when passing through the soil, thus it is requires periodic checking and should be disinfected when used for mass consumption (Sasakova *et al.*, 2018). In Tanzania, scarce water resources and poor sanitation make people more vulnerable to outbreaks of cholera and other waterborne diseases (Mboera *et al.*, 2011). It has been reported that among the notifiable diseases, water borne diseases account for 23 900 deaths per year and the most affected people be the children under 5 years of age (Elisante and Muzuka, 2016). Despite causing deaths, it is

also reported that water-related diseases prevent people from living active lives as well as working (Memon, 2011). Therefore, poor supply of safe drinking water, basic sanitation and hygienic practices is highly associated with high morbidity and mortality from feacal-orally transmitted diseases.

The presence of *Escherichia coli* and Enterobacter species in water is considered as a possible indicator of the presence of pathogens like *Clostridium pefringens*, *Salmonella*, and protozoa. However, *E. coli* is considered to be the most superior indicator of feacal contamination in drinking water which is abundant in human and animal faeces (Edberg *et al.*, 2000). Consumption of contaminated water can cause illnesses like diarrhea, dysentery, and gastroenteritis to infants, young children, and the elderly (Bharadwaj and Sharma, 2016). *E. coli* compromise the safety and quality of the water consumed by people worldwide (Lukubye, 2017).

In developing countries, illness and mortality due to waterborne salmonellosis has been reported to have increased (Lyimo *et al.*, 2016). In Tanzania, in 2012, about 20% of the reported diseases were attributed to typhoid fever alone (Mwang'onde *et al.*, 2013). Therefore, assessing the microbial quality in water sources especially those accessible by community is of great concern so as to ensure that the community consumes safe water. This study is also undertaken because there is limited information on microbiological parameters of stream and borehole water in Lushoto district, Tanzania.

3.3 Material and Methods

3.3.1 Study area

This study was carried out in Lushoto district, in Tanga region. Samples were obtained from two streams; Shaghai and Daa in Mbaru and Sunga wards respectively. Borehole

water was also obtained from the same wards. Lushoto district is situated in the northern part of Tanga Region. It lies between latitude 4°25 and 4°55'S, and longitude 30°10 and 38°35E. It is one of the eight districts of Tanga Region, with a total area of 4092 Km² (URT, 2013). The main sources of water for the district are springs, streams and boreholes, where streams flow down the slopes of Usambara Mountains (URT, 2013). Previously, these streams were flowing throughout the year but recently the volume of water tends to decrease during the dry season (July to September). Changes in water quantity were attributed by replacement of natural forests by pine plantations as well as deforestation. Lushoto district has been selected as a study area due to the fact that communities depend on streams as well as boreholes as sources of water for drinking and other domestic purposes.

3.3.2 Materials

Materials used for this study were water from boreholes and streams found in two wards. Other materials included measuring cylinder, weighing balance-Model PL202-S (Mettler Toledo, USA) distilled water, filtration system-Bio vac Model 331/631 (Rocker scientific, India), micro filter 0.45um, petri dishes, bottles (glass and plastic) and Incubator- Memmert (Fisher scientific, German).

3.3.3 Study design

Cross sectional design was used in this study. Samples for microbiological parameters (*E. coli* and *Salmonella*) were drawn from three points of each stream: forest, populated with agricultural activities, less populated with agricultural activities. Cross sectional design was also applied to boreholes water.

3.3.3.1 Sampling plan and data collection

Purposive sampling plan was used in this study to collect samples from boreholes and stream found in two wards in Lushoto district. Sampling was carried out during the dry season from November to December 2018. Samples were obtained from two streams; Shagayu in Mbaru ward and Daa in Sunga ward. Borehole water was also obtained from the same wards. A total of 24 samples were collected from the two sources of water. Water samples from the streams and boreholes were collected in the morning in a transparent clean and 500 mL sterile autoclavable glass bottles. Water sample from stream was collected in duplicate at three points from each village (a total of 12 samples which analyzed in triplicate to make a total of 36 data for each parameter) based on the presence or absence of agricultural activities and/or settlement along the water streams in Mbaru and Sunga wards. Likewise water samples from the borehole were also collected in duplicate from the three boreholes found in each ward and analyzed in triplicate for each parameter. Before collection the tap/nozzle was sterilized with cotton wool soaked in 70% v/v ethanol and allowed to run for three minutes. Samples were stored and transported in an insulated box maintained at 00 to 40 C and transported to Tanga water laboratory for microbiological analysis.

3.3.4 Microbiological analyses

3.3.4.1 Detection and Enumeration of Escherichia coli

Enumerations of *Escherichia coli* in borehole and stream water samples were determined according to ISO 9308-1:2014 by using membrane filtration. Results were expressed in CFU/100mL.

3.3.4.2 Detection of Salmonella species

Salmonella was determined according to standard operating procedure at Tanga water laboratory developed from EPA. Results were expressed in CFU/100mL.

3.3.5 Statistical analysis

Nested design was applied using the following model

$$Y_{ijk} = \mu + \beta_j + \alpha_{(j)i} + \varepsilon_{ijk}$$
 and $Y_{ijk} = \mu + \lambda_k + \rho_{(k)\chi} + \varepsilon_{ijk}$

Where by:

 Y_{ijk} = Dependant variable, μ =General mean, β_j = 1, 2, (stream),

 $\alpha_{(j)i} = 1, 2,3$ (effect of location nested within stream), $\lambda_{k=1,2(ward)}$,

 $\rho_{(k)x}$ = (effect of borehole nested within the ward), and ϵ_{ijk} = Random error

Data was analyzed using R- statistical package software. Nested design was applied on the stream and boreholes water to determine the effect of location nested within a stream and effect of boreholes water in the wards. Analysis of Variance (ANOVA) was carried out to determine the significant difference between the location within the stream and boreholes water between the wards. Means were separated using Tukey's Honest at p<0.05.

3.4 Results and Discussion

3.4.1 Microbiological parameters

3.4.1.1 Location nested within the Streams and between the streams

The microbiological parameters from the streams studied are presented in the following sections. Specifically, the results summarize the Mean value for *Escherichia coli* and *Salmonella* spp which were expressed in CFU/100mL. The results for the location nested

within streams studied are presented in Table 2.1. Likewise, comparison of the two streams in terms of microbial load (*E. coli* and *Salmonella*) were also elaborated

Table 3.1: Mean colony count of E. coli and Salmonella found in Daa and Shagayu streams

		Parameters	
Stream	Locations	E. Coli, cfu/100ml	Salmonellae, cfu/100ml
Daa	1A0	1.67 ± 0.52^{b}	$*0.00 \pm 0.00^{a}$
	2A1	9.33 ± 1.63^{a}	$7.67 \pm 1.51^{\mathrm{b}}$
	3A2	10.33 ± 2.34^{a}	7.33 ± 1.63^{b}
Average		7.11±4.28	5.00±3.83
Shagayu	1B0	$*0.00\pm0.00^{b}$	$*0.00\pm0.00^{a}$
	2B1	18.00 ± 1.79^{c}	11.00 ± 2.09^{c}
	3B2	7.67 ± 1.51^{a}	1.67 ± 0.52^{a}
Average		8.56±7.69	4.61±4.47
TZS 789		Absent/100mL	Absent/100mL
WHO 2011		Absent/100mL	Absent/100mL

Values in the same column having the same superscript letters are not significantly different (p > 0.05) (Tukey's Honest)

1A0-Forest for Daa stream

2A1-Kwamamkoa (Agricultural with population), 3A2- Komboheo (Agricultural and less population)

1B0-Forest from Shagayu stream

2B1- Ludende (Agricultural with population), 3B2- Kumbamtoni (Agricultural with less population)

3.4.1.2 The effect of location on stream contamination by E. coli

Escherichia coli (E. coli) is a member of total coliform group of bacteria that is found only in the intestines of mammals, including humans and animals. The presence of E. coli in water indicates recent fecal contamination and may also indicate the possible presence of disease-causing pathogens, such as bacteria, viruses, and parasites.

^{*}complied with standards

E. coli was one of the microbial parameter tested in this study. Results obtained revealed that 83% of the samples collected from the two streams (Shagayu and Daa) within three locations (forest, agricultural with population area and agricultural and less populationarea) were contaminated with E. coli and only 17% of samples were free from E. coli.

Furthermore, the mean results for E. coli obtained from two streams ranged from 0- 18.00±1.79 Colonies per 100mL (Table 3.1). Significant difference (p<0.05) in E. coli contamination was observed in three locations (forest, agricultural with population and agricultural with less population). Sample collected from forest in both streams showed no significant difference (p>0.05) in E. coli contamination. However, minimal detection of E. coli was observed in sample collected from the forest in Daa stream while there was no detection for E. coli in samples from forest in Shagayu stream. Non detection of E. coli observed at the source (forest) confirms lack of human activities/settlement and animals which could contribute to fecal (E. coli) contamination. The minimal detection of E. coli at the source in Daa stream might be associated with wild animals which could defecate directly into water bodies and pollute water. Similar observation was also reported by (Guber et al., 2015; Graves et al., 2003; and Somarell et al., 2007). Detection of E. coli at the forest in this study corroborate with previous study conducted by Goto and Yan (2009) whose finding reported E. coli contamination in Manoa stream, Hawaii which was adjacent to the forest. Likewise Garcia-Armisen and Servais (2007) reported mean value of E. coli 39CFU/100mL in stream water of Seine river, in France which is flowing through forest area. Furthermore, it was observed that in both streams, samples collected from the populated area with agricultural activities were detected with E. coli however the microbial load (E. coli) at Ludende in Shagayu stream was twice to that

observed at Kwamamkoa which was both agricultural area with population. Contamination at these areas might due to poor water management and exposure to contamination from human or animal wastes. In addition, the behavioral and hygienic practices of the community members might also be the contributing factors.

During the survey, it was observed that communities in the study area used stream water for bathing and washing clothes which would eventually contribute to water contamination. Also application of cattle manure was observed in farmers near both streams. This could also contribute to the presence of *E. coli* to the nearby stream since cattle are commonly considered as a principal reservoir of *E. coli*. This observation is also supported by Johnson *et al.* (2003); and Aitken (2003). Previous study reported by Davies-Colley *et al.* (2004) found high concentrations of *E. coli* in stream water of Sherry River in New Zealand which was near agricultural area. In addition the finding reported by Garcia-Armisen and Servais (2007 in stream water of Seine river which was adjacent to agricultural area indicated high number of *E. coli* with mean value of 47CFU/100mL.

Moreover water samples collected from Komboheo and Kumbamtoi which was agricultural area with less population were detected with of *E. coli*. Contamination of water by this pathogen in these areas was not surprising since it involved in crop cultivation and surrounded by few human settlements. Therefore, *E. coli* could be attributed by discharge of livestock feacal waste and other sewage wastes from the settlements. Comparing the mean value of *E. coli* in both streams, it showed that both streams were contaminated with *E. coli* as indicated in Table 3.1. However, with exception of samples collected from forest in Shagayu stream, the average concentration of *E. coli* at three locations in two streams did not comply with neither the Tanzania

Standard (TZS, 2016) nor the WHO (2011) which state that *E. coli* should not be detected drinking water sample. Therefore with regard to *E. coli*, water from both streams are not safe for human consumption.

3.4.1.3 The effects of location on stream contamination by Salmonella

In this study, the presence of *Salmonella* was also tested as an important microbiological parameter. Results obtained from the two streams ranged from 0-11 CFU/100mL. This revealed that, 33% of samples tested in two streams were free from salmonellae and these had been collected from the forest, while 67% of samples detected in the rest of locations. Results obtained indicated significant differences (p<0.05) in *Salmonella* in three locations (forest, agricultural with population and agricultural with less population) of the streams. however there was no significant differences (p>0.05) in *Salmonella* in water samples collected from the forest in both streams and agricultural with less population (Shagayu streams), but minimal detection was observed at Kumbamtoni (agricultural with less population). *Salmonella* was not detected in samples from the forest in both streams due to lack of human activities which could influence water contamination by *Salmonella*. Moreover the minimal detection of *Salmonella* at Kumbamtoi might be associated with application of organic manure which is released into nearby stream due to irrigation practices done by farmers.

As observed in this study that samples collected from agricultural with population in both streams were contaminated with *Salmonella*; Ludende was highly contaminated. This result may be caused by sewage discharges from the household and application of organic manure to farms. This was also supported by Patchanee *et al.* (2010) who indicated that 58.8% of water sample collected at different streams of white Oak river, USA which was near residential area and 50% near agricultural activities were

contaminated with *Salmonella*. Other observations regarding *Salmonella* contamination in various streams due to agricultural activities were reported by Walters *et al.* (2011); Johnson *et al.* (2003); Poma *et al.* (2016) in California, Bolivia and Canada respectively. With regard to *Salmonella* in both streams which was above the required limit by TZS (2016) and WHO (2011), water from these streams are not safe for human use.

3.4.1.4 Prevalence of E. coli and Salmonella among the borehole water found in two wards

The study results for the microbiological parameters from the six boreholes studied between the two wards are presented in Table 3.2. These results summarize the mean colony counts for *Escherichia coli* and *Salmonella* which were expressed in CFU/100mL.

Table 3.2: The mean value for E. coli and Salmonella in borehole water found in two wards

Ward		Parameters	eters			
	LOCATION	E. coli (CFU/100mL)	Salmonella(CFU/100mL)			
SUNGA	Alufea	ND	ND			
	Madukani	*2.00±0.63 ^a	ND			
	Kwemashu	ND	ND			
MBARU	Ludende	ND	ND			
	Masereka	ND	ND			
	Chambogo	ND	ND			
	TZS 2016	Absent/100mL	Absent/100mL			
	WHO 2011	Absent/100mL	Absent/100mL			

Values in the same column having the superscript letters are significantly different (p < 0.05) (Tukey's Honest)

CFU-Colony Forming Unit

ND-Not detected

The mean results obtained for *E. coli* varied from not detected to 2.00±0.63CFU/100mL. Out of all boreholes water studied in the two wards *E. coli* was detected from only one location which was Madukan in Sunga ward. The presence of *E. coli* in water samples collected from Madukani was of great concern as it implied feacal contamination.

The presence of *E. coli* in borehole water might be attributed by close proximity of open hole which was contaminated with animal feaces and other wastes. The hole was previously used as a source of water before the borehole became operational. In addition, farming activities were observed in the area during sample collection which involved application of organic fertilizer. All these could seep into the soil and end up in the borehole. The same observation was also reported by Palamuleni and Akoth (2015).

^{*}Failed to meet standards

Furthermore, other researchers argued that the presence of rusty pipes used in water distribution might allow seepages of microbial contaminants into the borehole (Uzoigwe and Agwa, 2012; Adogo *et al.*, 2016). There are several studies which indicated *E. coli* contamination in borehole water which includes Mpenyana *et al.* (2012) in South Africa; Bashir *et al.* (2018) in Nigeria; Obioma *et al.* (2017) in Nigeria; Izah and Ineyougha (2015) in Nigeria; Lutterodt *et al.* (2018) in Ghana; Takal and Quaye-Ballard. (2018) in Ghana and Bekuretsion *et al.* (2018) in Ethiopia.

Other studies that has supported similar finding were Ukpong, and Okon (2013) in Nigeria; Uzoigwe and Agwa (2012) in Nigeria; and Thani *et al.* (2016) in Mombasa with the value of 18.75%, 14.3% and 65.8% respectively. The presence of *E. coli* in drinking is a risk to public health since this bacterium causes human illness such as diarrhea in both children and adults which is latter characterized primarily by bacteria attacking intestinal epithelia (Landeros-Sánchez *et al.*, 2012). Furthermore water sample collected from most of the boreholes were not detected with *E. coli*. This may probably due to the fact that these boreholes were located far from the toilet. Another reason might be associated with the holes which were drilled deep down hence clean water was available in the boreholes. The same observation was also discussed by Obioma *et al.* (2017).

The finding of this study in term of free contamination of *E. coli* in boreholes was also supported by Isa *et al.* (2013) in Nigeria. In addition, the finding by Bello *et al.* (2013) in Nigeria; and Kanyerere *et al.* (2012) in Malawi did not detect *E. coli* in boreholes tested. Comparing the wards in terms of *E. coli* contamination in boreholes, Mbaru ward was free from contamination and suggested to be safe for human use while in Sunga ward borehole water from Madukan was not safe for human use since it exceeded the limit stated by TZS (2016) and WHO (2011) as indicated in Table 3.2.

Salmonella was also tested as a parameter of interest in this study. Results indicated that none of samples collected from boreholes in both wards were contaminated with Salmonella. This might be associated with the location of these boreholes which were not in close proximity to the toilet, sewage or discharge from septic tanks. Furthermore, the absence of Salmonella might also be attributed by the depth of boreholes. The other reason might also associated by the nature of the soil which does not allow the transfer of bacteria to the groundwater. This observation is also discussed by Obafemi et al. (2018). The finding of these results are contrary to various studies by Izah and Ineyougha (2015) in Nigeria; Palamuleni and Akoth (2015) in South Africa; Onwughara et al. (2013) in Nigeria; Takal and Quaye-Ballard (2018) in Ghana, who found Salmonella contamination in borehole water. Salmonella contamination in boreholes might be attributed by closeness of toilet, sewage system, and waste dumps to the water source.

A finding by Nwandkor and Ifeanyi (2015) in Nigeria indicated that out of 50 borehole water samples tested for *Salmonella* only one sample was contaminated. The author observed that presence of *Salmonella* in one borehole might be associated with the shallow depth of the borehole. Comparing the wards, boreholes found in both wards were free from *Salmonella* hence complied with TZS (2016) and WHO (2011). Therefore it is concluded that all the borehole water are safe for human consumption as far as *Salmonella* is concerned.

3.5 Conclusion

The situation of water quality and safety in the study area is in serious need of improvement, since there is a huge indication of water contamination by *E. coli* and *Salmonella* in both streams especially in agricultural areas (both populated and less populated). Contamination of water is associated with poor agricultural practices and poor

hygienic condition among the community members especially in populated areas located upstream. This might cause transportation of wastes down the streams (agricultural area with less population). Therefore people consuming water downstream are also at risk of waterborne disease hence there is a need of preserving the water sources so as to rescue community member living near agricultural area. Furthermore the quality of borehole may not always be of pristine as is perceived since it was revealed that the borehole at Madukani was contaminated with *E. coli*. This gives an alert that water from both sources are not safe for use without treatment. Therefore education to the community members should be given indicating—the possible health risks associated with consumption of water from the two sources without treatments such as boiling so as to prevent waterborne diseases and ensure their wellbeing.

3.6 Recommendation

Based on the key findings of this study, the following are the recommendations made to community members in the study area, and various water stakeholders (such as policy makers, and community health officers) so as to improve the quality of both streams and borehole water used by community members in the District.

i. Introduce community based water management systems where community are involved in the decision making process so that they take responsibility in ensuring that stream and boreholes are kept clean and safe at all times This will help to ensure good management of water sources through community committees that can initiate regulations which govern activities carried out along the water sources such as agricultural activities, construction of pit latrine near to water sources and general hygiene such as prohibiting bathing, washing clothes near or into the stream water.

ii. Introduce policies on water sources that should consider the role of community members in conserving the water resource. Traditional knowledge and /or perceptions of the community on water conservation can help to have a national water management plan or framework which can be integrated with modern water management systems. Policies governing land resource and environment as a whole should take into consideration local community knowledge on the issue of conserving land and environment, so as to avoid pollution that can consequently affects water, and eventually health of the consumers (people and animals).

Generally, the water policy in collaboration with the health policy should look at rapid measures or precautions to be taken before the problem of water contamination becomes more critical.

Moreover, recommendations on areas for further studies need to focus on a number of aspects.

The following are interesting areas of studies in the future:

- More microbiological parameters to be tested as per TZS 789.such parameters includes Streptococcus, Staphylococcus aureus, Shigella and total coliforms
- The same study should be conducted in other rural areas of Tanzania or elsewhere
 where majority depend on surface water such as stream water for their
 consumption,
- The same study can be undertaken during the rainy season or both seasons.

 The purpose is to make comparison between the two seasons and see if there are significant similarities or differences between the two seasons.

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CHAPTER FOUR

4.0 Overall Conclusions and Recommendations

4.1 Conclusions

From a water quality and safety point of view, results for the chemical parameters in streams and boreholes found in Sunga and Mbaru wards indicated tolerable quality as per TZS except phosphate from both streams which was high at Kwamamkoa and Ludende (agricultural with population) and Komboheo (agriculture and less population). However microbiological parameters tested indicated that both streams near agricultural area with more population and agricultural with less population were contaminated with *E. coli* and *Salmonella*. Therefore community engagement through improving water quality such as good agricultural practices and good hygienic practices in both streams are very essential to ensure that they rescue community from consuming contaminated water. Also education on how to treat water before use should be provided so that people become aware on risk of consuming untreated water. Moreover, microbiological quality of boreholes was satisfactory except from one borehole located at Madukani which was contaminated with *E. coli*. General results showed that borehole water was more safe compared to stream water.

4.2 Recommendations

Based on the findings of this study the followings are recommended to the people:

 The same study to be conducted during the rainy season or both seasons for the purpose of comparing the findings whether there are similarities or differences between the two seasons.

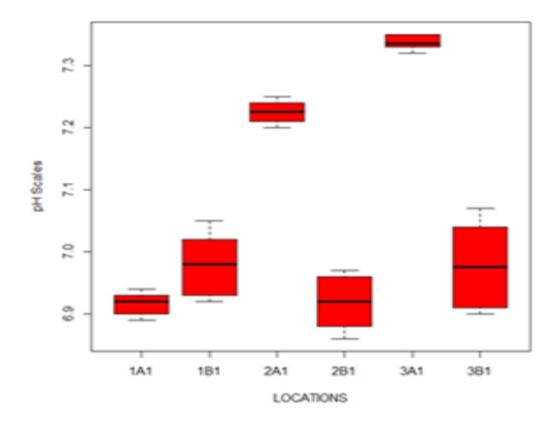
- ii. More parameters to be assessed as per TZS 789. These include sulphate, fluoride, chloride, copper, conductivity, turbidity, biochemical oxygen demand, total suspended solid *Streptococcus*, *Staphylococcus aureus*, *Shigella and total coliforms* and pesticides. However information on the pesticides used in the study area is very crucial so that residue will be assessed based on the pesticide in use.
- iii. Water should be treated regardless of its source to improve its quality and safety for human consumption.
- iv. The same study should be conducted in a rural area of Tanzania where community depends on surface water such as stream water

APPENDICES

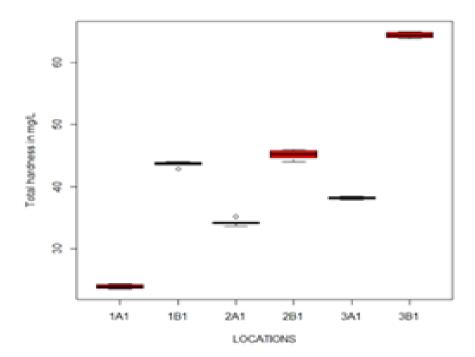
Appendix 1: Boxplot showing the mean value for chemical parameters within the location of the two streams (n=36).

1A1-Forest for stream A, 2A1- Kwamamkoa (Agriculture with population), 3A1- Komboheo (Agriculture with less population),

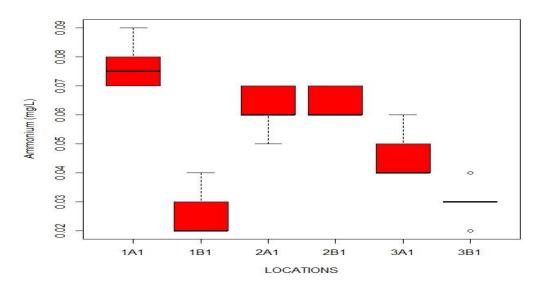
1B1-Forest for stream B, 2B1- Ludende (Agriculture with population), 3B1- Kumbamtoni (Agriculture and with less population).



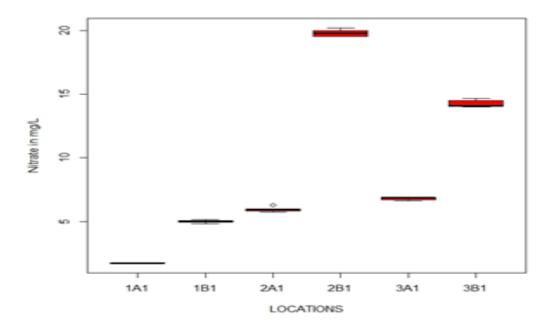
Boxplot showing the mean pH values between three locations of the streams (n=36)



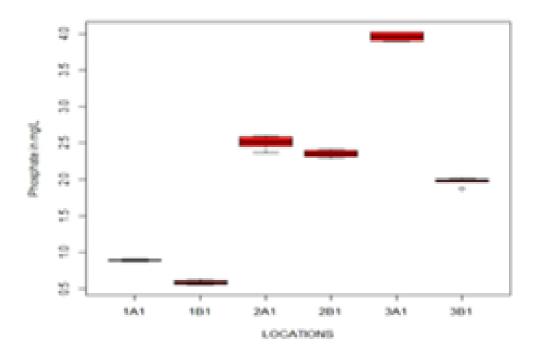
Boxplot showing the mean values for total hardness between three locations of the streams (n=36).



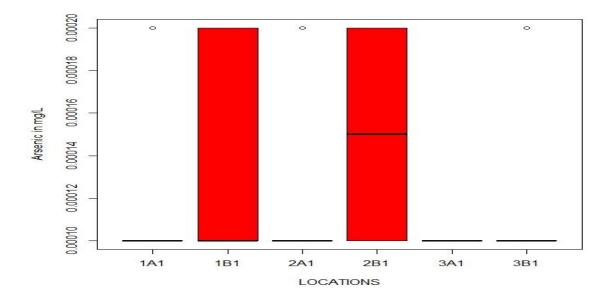
Boxplot showing the mean concentration of ammonia between three locations of the streams (n=36



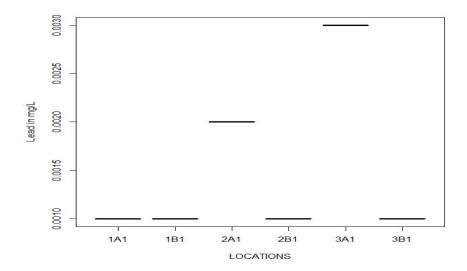
Boxplot showing the mean concentration of nitrate between three locations of the streams (n=36)



Boxplot showing the mean values of phosphate between three locations of the streams (n=36)



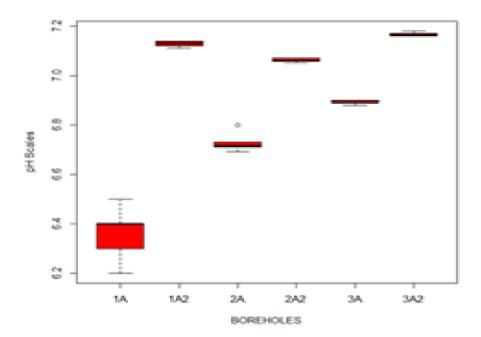
Boxplot showing the mean values of arsenic between three locations of the streams (n=36)



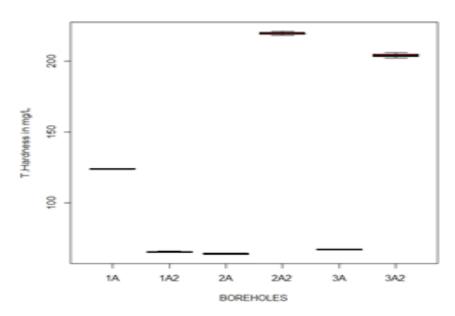
Boxplot showing the mean values for total hardness between three locations of the streams (n=36)

Boxplot showing the mean values of chemical parameters for borehole in two wards and comparing the wards (n=36). 1A-Alufea, 2A-Madukani, 3A-Kwemashui,

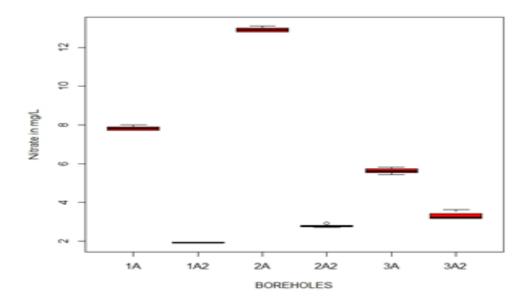
1A2-Masereka, 2A2-Ludende, 3A2-Chambogo



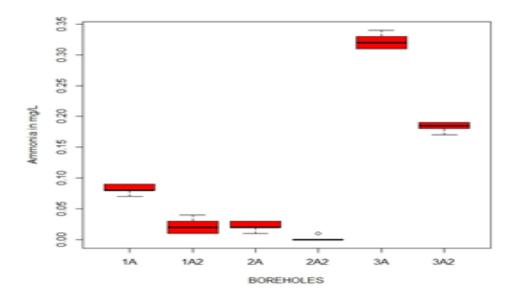
Boxplot indicating the mean pH value among the boreholes found in two wards (n=36)



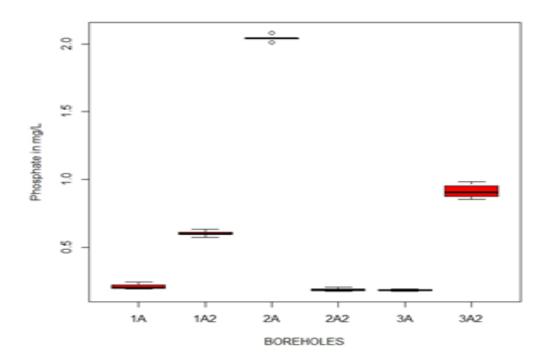
Boxplot showing the mean concentration of total hardness among the boreholes found in two wards (n=36)



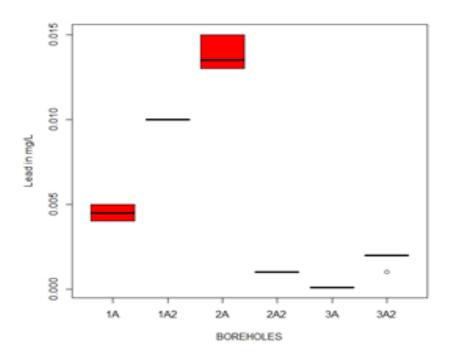
Boxplot showing the mean concentration of nitrate among the boreholes found in two wards (n=36)



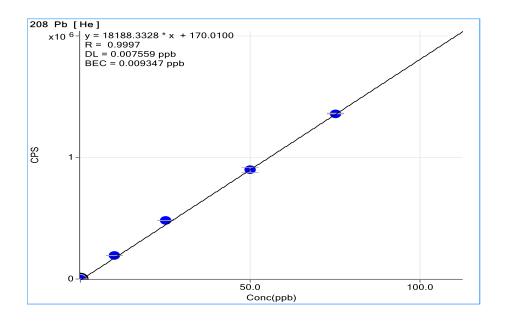
Boxplot showing the mean concentration of ammonia among the boreholes found in two wards (n=36



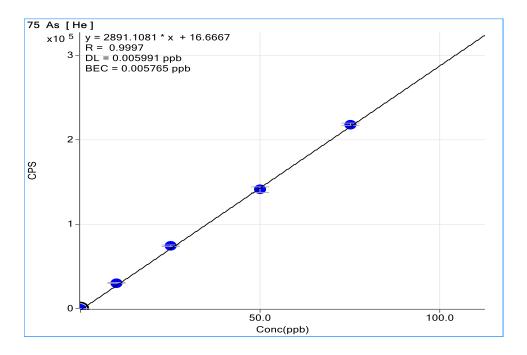
Boxplot showing the mean concentration of phosphate in boreholes found in two wards (n=-36)



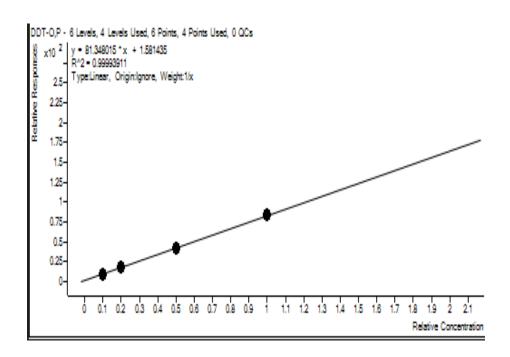
Boxplot showing mean concentration of lead (Pb) in boreholes found in two wards (n=36)



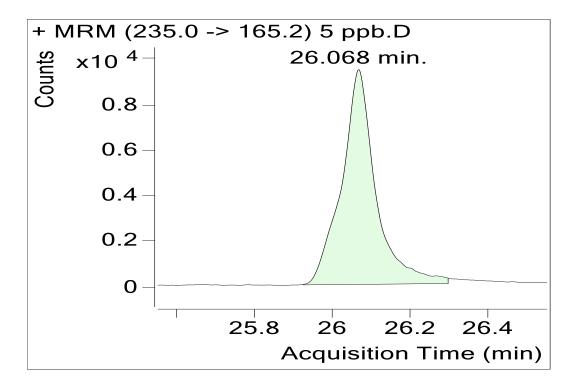
Calibration curve for lead (Pb)



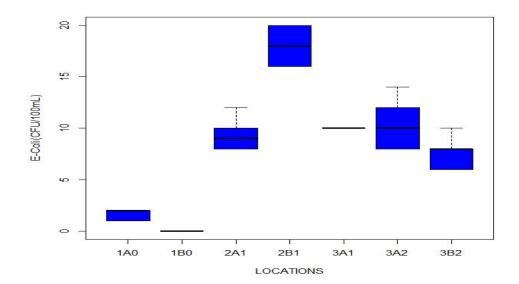
Calibration curve for arsenic (As)



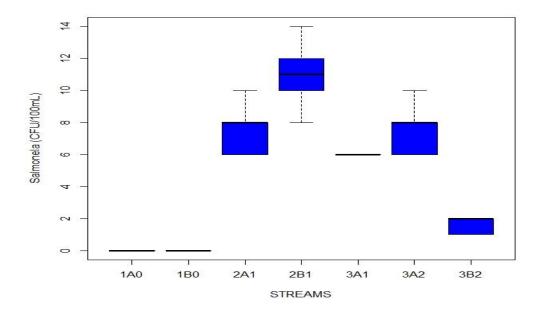
Calibration curve for DDT



Chromatogram for DDT



Boxplot showing the mean results of *E. coli* within three locations in two streams



Boxplot showing the mean value of Salmonella within three locations of the two streams (n=36)