CONTAMINATION HEALTH RISKS OF PARASITIC HELMINTHS ASSOCIATED WITH REUSED WASTEWATER FOR VEGETABLE IRRIGATION IN MBEYA, TANZANIA

VENANCE THEOPHIL MSOFFE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF A MASTER OF SCIENCE IN MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2019

ABSTRACT

Reuse of wastewater for vegetable irrigation is common in developing countries due to its accessibility and being rich in fertilizing elements thus improving crops yield. Regardless of these advantages, the practice plays an important role in transmission of intestinal parasitic helminths to vegetable consumers. This study was conducted to assess farmers' awareness, perceptions, managerial and consumption practices regarding vegetables irrigated with reused wastewater on health risks of consumers. In addition, this study assessed intestinal parasitic helminths eggs concentration and profile from samples of wastewater and vegetables irrigated with wastewater. Sociological survey data were collected from 61 purposively selected smallholder vegetable farmers using semi-structured questionnaire. A total of 56 samples of effluent water from wastewater treatment ponds (WWTP), polluted stream water (PSW) and reused wastewater irrigated vegetables were collected for parasitological examination. Parasitological examination was conducted using the floatation method adopting a modified Bailenger protocol with zinc sulphate (1.3 specific gravity). Then all Taeniid eggs positive isolates were subjected to PCR for *Taenia solium* detection. Differences in farmers' awareness and perception levels were analyzed by multivariate analysis of covariance (MANCOVA). The association between demographic factors and vegetable consumption practices were analyzed using multivariate linear regression analysis model. The results indicated presence of risky practices for transmission of intestinal helminths in the study area such as consumption of raw vegetables. Education levels of the respondents found to influence consumption practices of vegetables irrigated with wastewater ($P<0.05$) whereby more educated individuals were not certain on safety of the water. Besides, the analysis of parasitic helminths data showed that, 88% of WWTP, 78.6% of PSW and 75% of the vegetables were contaminated with intestinal parasitic helminths eggs with mean concentration of 1.14 eggs/L, 0.667 eggs/L and 3.3 eggs/100g, respectively. Intestinal parasitic helminths found were: *Ascaris sp.,* hookworms, *Taenia sp., Trichuris sp., Strongyloides sp.* and *Enterobius sp*., and *Hymenolepsis sp*. Polymerase chain reaction analysis revealed that 26.7% of samples were contaminated with *T. solium* eggs. It is concluded that polluted streams channeled with effluent water from Kalobe (WWTPs) is contaminated with high levels of intestinal parasitic helminths eggs than what recommended by World Health Organization for unrestricted agriculture irrigation. Furthermore, molecular analysis confirmed involvement of *T. solium* in some isolates of Taeniid eggs. Thus, it is recommended that, community training particularly in health risks associated with contamination of crop irrigated using recovered wastewater should be considered. More studies should be conducted on reused wastewater chemical contaminations and other microbial pathogens profiles using modern techniques such as molecular methods.

DECLARATION

I, VENANCE THEOPHIL MSOFFE, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work, done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

Venance Theophil Msoffe Date

MSc. MBB Candidate

The above declaration is hereby confirmed:

Dr. Ernatus Mkupasi Date

(Supervisor)

Prof. Gerald Misinzo Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

Above all, my extremely grateful thanks go to the Almighty God who generously enters into my day-to-day process of preparing this work from its genesis to its completion. I owe a depth of gratitude to my Supervisors Dr. Ernatus Mkupasi and Prof. Gerald Misinzo of College of Veterinary Medicine and Biomedical Sciences at Sokoine University of Agriculture (SUA) and Prof. Maria Vang Johansen of Copenhagen University, Denmark, for their tireless positive assistance and support, and also for their inspirations, advice, perseverance, and encouragements throughout my course of this research. Equally, I appreciate the ancillary concern and back-up of Mkwawa University College of Education (MUCE), the primary sponsor of my Masters studies and Prof. Maria Vang Johansen (from University of Copenhagen, Denmark) under SOLID project funded by the European and Developing Countries Clinical Trials Partnership (EDCTP) who sponsored research component of this work. I extend forth my sincerely thanks to vegetable farmers in Mbeya for sharing their knowledge and experience about reused wastewater irrigation. I also wish to acknowledge the unreserved support of laboratory technical staff at Mbeya Zonal Referral Hospital department of Parasitology and Sokoine University of Agriculture Department of Microbiology, Parasitology and Biotechnology where this work was conducted. Once again I owe a great deal to my family for their support in prayers and encouragement they provide to me. Also, I am really grad to convey my thanks and appreciations for the contributions and assistance of my friends and colleague Michael Martin, Claus Thomas and Emil Thomas, may Almighty God grant their prayers.

DEDICATION

I wish to dedicate this work to my father, Theophil Msoffe and my mother, Mary Mtalimboto whom I owe my early cognitive development, but, without forgetting, my beloved wife Lilian and my lovely daughter Rosemerry.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS, ACRONYMS AND SYMBOLS

BLS	Bonde la songwe
CPW	control polluted water
DNA	deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
ITD	Itende
KLB	Kalobe
Ltd	limited
MANCOVA	multivariate analysis of covariance
MBM	modified Bailenger method
MIGA	major income generating activity
MSH	Mshewe
NSG	Nsalaga
NSL	Nsalala
NTD	Neglected Tropical Diseases
PCR	polymerase chain reaction
PSCW	polluted stream channelled with wastewater
Sig.	Significant level
STH	soil-transmitted helminths
TBS	Tanzania Bureau of Statistics
URT	United Republic of Tanzania
US EPA	United States Environment Protection Agency
UTU	Utengule usongwe
WHO	World Health Organization
WWTP	waste water treatment pond
Λ	lambda

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Reuse of wastewater for agricultural irrigation is increasingly becoming common due to its abundance in fertilizing elements for better growth and survival of crops and thus, lessening the requirement of commercial fertilizers (Jiménez, 2006; Jimenez and Asano, 2012). Also due to its easy accessibility and availability, it is used to remedy challenge of water scarcity for different human activities in various countries (Mara and Cairncross, 1989). Recovered wastewater channeled from urban or rural areas are reported to be laden with full spectrum of excreted bacterial, viral, protozoan, and helminthic disease pathogens reflecting the carriage and infection status in that particular community (Hajjami *et al.*, 2012).

Crops irrigated with reused wastewater is associated with high risk of transmission of disease through consumption of crops like vegetables irrigated with contaminated recovered wastewater (Nwele *et al*., 2013; Sabbahi *et al.*, 2018). Health practitioners encourage eating of vegetable crops as part of nutritional diet to enhance health status, however, various studies reported an increase of food-borne related infections which have direct association with eating of fresh vegetables (Crompton and Nesheim, 2002; Ensink *et al.*, 2002). Individuals involved in the use of recovered wastewater may be the direct victim of the associated risk, though, this may indirectly involve large community through consumption of vegetables sold in the markets far away from farming places (Amoah *et al.*, 2007; Loganathan *et al.*, 2016). In general, both producers and consumers of crops irrigated with reused wastewater are at risk of acquiring waterborne pathogens such as intestinal parasites.

Diverse factors may influence transmission of parasitic helminths through reclaimed wastewater such as deprived sanitation and hygienic practices. Also poor latrine system constructed alongside and channeled to rural water sources, contaminates water which may be used for drinking or irrigation in peri-urban or rural areas and thus transmit whatever profile of parasitic helminths pathogens to the community involved (Annepu, 2012). However, poor management of municipals sewerage systems may contribute significantly to the risk rate of intestinal parasitic disease pathogens in peri-urban and rural areas in various regions of developing countries (Okoh *et al.,* 2010; Adegoke *et al*., 2018). Unrestricted reuse of raw or effluent water from wastewater treatment ponds (WWTP) for different human activities including agriculture crops irrigation, washing, and for cooking influence transmission cycle of various infective stages of parasites such as parasitic helminth eggs (Ensink *et al.*, 2008).

The risk of transmission can be low or high depending in efficient and effectiveness of WWTPs and infection status of a particular community (Tilley *et al*., 2014). Different reports avails that municipal sewerage systems are not 100% efficient in elimination of parasitic helminths eggs (Rahmatiyar *et al*., 2014). Therefore the resulted reclaimed water is not complying with World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) requirement of <1egg/L of parasitic helminths in wastewater to be used for unrestricted agriculture (Stott *et al*.,2003; Okojokwu *et al*., 2014; Sabbahi *et al*., 2018). The recovered water from WWTPs are channeled in peri-urban and rural streams or allowed to drain to nearby rivers. Downstream the water is used for various human activities including irrigation of vegetable fields and even drinking in some places (Jaramillo and Restrepo, 2017). This suggests that if the recovered water from WWTPs contains microbial or infective parasitic disease pathogen they contaminate irrigated crops. Depending to mode of transmission of disease agents they may infect farmers directly, for example cutaneous larva migrants of *Stronglyloides stercolaris* or indirect through eating contaminated crops (Nutman, 2017).

Soil-transmitted helminths (STH) such as *Ascaris lumbricoides*, Hookworm species, *Trichuris trichiura* and *Stronglyloides sp*. and other important food borne parasitic helminths such as *Taenia solium* which play a great role in risk of contaminating irrigated crops due to their long viability in polluted en vironmental sources. de Silva *et al.* (2003) reported that globally approximately more than 1 billion people are infected by *A. lumbricoides*, 795 million are infected by *T. trichiura* and 740 are infected by *Ancylostoma duodenale* and *Necator americanus*.

Pathogenic parasites have a more infective risk compared to microbes (bacteria and viruses) since they can persist in wastewater for long time and remain viable (Scott, 2003). For example, viability of protozoa cysts in wastewater may reach about 30 days whereas, helminths eggs may remain viable in wastewater for more than 12 months (Gaasenbeek and Borgsteede, 1998). Moreover, infective helminth eggs require low dose to cause new infection to a new host such that, only few as one egg can be a causative agent to a particular helminthic disease (Jimenez, 2007).

In particular case, *T. solium* is among the utmost threatening parasitic cestode for most of developing countries. It is responsible for causing porcine cysticercosis and human Taeniasis/cysticercosis which may result into neurocysticercosis if the cyst situate into brain the condition associated to adaptive epilepsy in human (Garcia and Del Brutto, 2003). In 2010, *T. solium* cysticercosis was categorized among zoonotic neglected tropical disease (NTD) with the possibility of being eliminated (WHO, 2010). It is highly endemic in many countries of sub-Saharan Africa, South-East Asia and Latin America

(Goussanou *et al.*, 2014). The socioeconomic impact of cysticercosis is immense as it affects the health and livelihood of subsistence farming communities by causing neurocysticercosis in humans, reducing the market value of pigs and making infected pork unsafe for human consumption (Braae *et al.*, 2014; Coral *et al.*, 2014; Karamon and Cencek, 2018). Various studies have reported detection of Taeniid eggs from wastewater for irrigation and vegetables irrigated with reused wastewater (Hajjami *et al.*, 2012; Loganathan *et al.*, 2016). Presence of helminthic eggs in wastewater reclaimed from local sewage treatment systems and used in crops irrigation is a good indicator of community risks to the infections (Okojokwu *et al.*, 2014).

Majority of studies established that the risk factors for *T. solium* transmission includes; poor sanitation, including the lack of latrines, unsafe water, poor pig husbandry practices, inadequate meat inspection and lack of knowledge about the causative agent (Mkupasi *et al*., 2011; Mwanjali *et al*., 2013). Neurocysticercosis results when parasite larvae lodge in the human brain, forming cysts. Interestingly, few studies had been conducted in Tanzania which reported cases associated to health risks of reused wastewater for crops irrigation, due to threat of contamination with different kind of disease agents such as parasitic helminths (Outwater *et al*., 2013; Ofred *et al*., 2016; Samson *et al*., 2017).

However, no extensive study have been documented regarding isolation and identification of Taeniid eggs from reuse wastewater and irrigated vegetables to the species specific levels. Full profile of selected helminths of public health importance in wastewater and vegetables should inevitably be known to ascertain the safety or risks to the community. Also in order to have relevant efficient and effective ways to control helminthic infections such as Taeniasis/cysticercosis in human, a full description of local risk factors for transmission regime is a prerequisite (Gabriël *et al*., 2017).

1.2 Problem Statement and study Justification

Despite of being important source of fertility contents in agriculture for crops production, wastewater poses a risk for transmission of biological agents of diseases such as viruses, bacteria, protozoa and helminths (Jaramillo and Restrepo, 2017). Little is known about sanitary and environmental risks associated with reuse of wastewater and wastewater irrigated vegetables in Tanzania. None has extensively addressed in particular the potential risk of reused wastewater and consumptions of irrigated vegetables contaminated with intestinal parasitic helminths eggs in peri-urban Mbeya city and Mbeya district.

The findings of this study avail intestinal parasitic helminths egg profile and their concentration from reused wastewater and irrigated vegetables in the study area. In addition the study will offer information on the direct link between reclaimed wastewater from WWTPs and irrigated vegetables parasitic helminths contamination. Hence, it will contributes significant information which will form a basis for formulating control measures to reduce or eliminate intestinal parasitic helminth infection in the community.

1.3 Objectives

1.3.1 Main objective

The main objective of this study was to determine the contamination health risks of intestinal parasitic helminths associated with reused wastewater for vegetable irrigations and molecular detection of *T. solium* from Taeniid eggs isolates in Mbeya, Tanzania.

1.3.2 Specific objectives

The specific objective of this study are;

i. To assess farmers' awareness, practices and perception regarding reused wastewater for vegetable irrigations,

ii. To identify intestinal parasitic helminths eggs recovered from reused wastewater and alongside irrigated vegetables, and

iii. To detect presence of *T. solium* in the Taeniid eggs isolates using multiplex PCR.

1.4 Hypothesis

H_1: Vegetable farmers are aware of pathogenic disease agents contamination of reused wastewater for vegetables irrigation.

H_2: Reused wastewater and irrigated vegetables are contaminated with intestinal parasitic helminths eggs.

H_3: There are *T. solium* eggs of diverse origins among Taeniid eggs identified from reused wastewater and irrigated vegetables.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Reused Wastewater and its risk for Agriculture Irrigation

2.1.1 Description

In this study "reused wastewater" is the phrase used synonymously with reclaimed wastewater to denote the out flowing of the water from wastewater treatment ponds (WWTPs) and being used again for various human activities including agriculture irrigation. Wastewater is the combination of wastes from various sources such as domestic, industrial, commercial or agriculture, and thus it can contain physical, chemical and biological pollutants (Tilley *et al.*, 2014). The WWTPs are ponds designed for treatment of wastewater through biodegradation of organic matter contents and remove pathogens using natural aggregates like ultraviolet light, temperature and sedimentation. The ponds consist of large man-made shallow basins comprising a single or several series of anaerobic, facultative and maturation ponds, of which the final pond is required to be safe for disposal into the environment (Kayombo *et al.*, 2005; WHO, 2006).

2.1.2 Wastewater irrigated crops and its risks

There are different ways the recovered wastewater can be reused such as agriculture irrigation, recharge of aquifers, seawater barriers, industrial applications, and other urban uses. Nevertheless, reused wastewater for crop irrigation is now a common practice in various developing countries including Tanzania. Globally, it is estimated that more than 20 million hectares of arable land are irrigated with wastewater (Bahri *et al.*, 2010). Also it was approximated that about 10% of population in African cities are engaged in urban and peri-urban agriculture using wastewater as source of water for irrigation (Drechsel *et al.*, 2006). Easy availability and accessibility of wastewater make it to be reliable source of water for agriculture irrigation in various cities of developing countries (Mojid *et al*., 2010; Mayilla *et al*., 2017; Samson *et al*., 2017). In one way reused wastewater for irrigation help to safeguard the agenda of food security, environmental conservation and sustainability of livelihood (Jiménez, 2006; Amoah *et al*., 2007).

Reuse of treated wastewater for vegetables and energy crops irrigation practices is encouraged by governments worldwide. It is a reliable and stable source of water that can save water scarcity and fertilizer costs challenges. Unlike clean water, the availability of wastewater do not vary with seasons of the year or climatic conditions, thus enabling farmers to grow crops throughout the year (Mojid *et al*., 2010). Moreover, wastewater contains nutrients that can influence well growth of crop and thus reduce cost of using manufactured fertilizer. Apart from strong governments support, reused wastewater irrigation practice faces several limitations, including social acceptance, environmental concerns and sanitation, and obstructive regulations that tend to hinder exploitation of its full potentials for development (Robinson *et al*., 2012; Mayilla *et al*., 2017; Samson *et al*., 2017). Additionally, public health threat of contamination of reused wastewater with pathogenic disease agents is the strong factor which insinuate for active management to prevent mankind.

Conducted studies have reported potential health risks associated with reused wastewater for crop irrigations to humans, animals and the environments. For instance, Feevale *et al*. (2012) reported the presence of enteric viruses and coliforms in Brazil. Also the study conducted to assess the parasitological profile in vegetables and fruits irrigated by reused wastewater in Nigeria reveal spectrum of various helminths eggs including *Schistosoma, Ascaris, Hymenolepsis, Enterobius, Trichuris, Diphybotrium and Taenia* species (Nwele *et al*., 2013). In Tanzania also effort has been made to assess health risks which may be associated with reused wastewater for agriculture purposes, for example, Mhongole *et al*. (2016) managed to recover *E .coli* bacteria from Mzumbe wastewater stabilization ponds in Morogoro, also study conducted by Outwater *et al*. (2013) in various parts of Tanzania unwrap the presence of various types of bacteria, protozoa and helminths in municipal wastewater systems which increase the risk of exposure to different kind of diseases. In fact, biological agents profile recovered from particular wastewater treatment system reflect infection of the local community which channel wastes to the treatment system (Carr *et al.*, 2001).

2.1.3 Wastewater health risks reduction

Wastewater treatment systems for pathogenic parasites removal range from the use of mechanical treatment procedures (which are conventional) to natural treatment procedures. In fact, majority of systems only treat parasites' infectious agents by separating them from wastewater suspensions into sediments or sludge while still active and viable (Stott *et al.*, 2003; Konaté *et al.*, 2013). Always the principle of sedimentation, filtration, predation, adsorption and absorption is associated with parasites removal in most wastewater treatment systems. In Africa, the most common wastewater treatment systems used includes WWTPs, activated sludge and constructed wetlands.

Depending to the expected uses of the end product of the system, these three wastewater treatment systems perform differently regarding efficiency of removal of parasites from wastewater. For example, WWTPs which is man-made coordinated series of anaerobic, facultative and maturation ponds perform wastewater treatment using ultraviolet light and coordinated activity of algae and heterotrophic bacteria, which function to remove pathogens and nitrogen (Pescod, 1992; Tilley *et al*., 2014). Constructed wetlands are man-made wetlands built to remove various types of pollutants present in wastewater that flows through these systems. They are constructed to refabricate the structure and function of natural wetlands. They are rich in microbial profile to influence the biochemical transformation of pollutants, they are biologically productive and most important, they are self-sustaining (Stott *et al*., 2003; Kayombo *et al.,* 2005*;* Outwater *et al.,* 2013). An activated sludge treatment system is a conventional technology of wastewater treatment process using aeration blowers and a biological floc composed of bacteria and protozoa (Tilley *et al*., 2014). Jemli *et al*. (2015) reported mean reduction efficiency of 99.7% and 95.36% for parasitic helminths and protozoa cysts respectively, by activated sludge in Tunisia which seem to be more effective compared to WWTPs. However, overall systematic analysis by Zacharia *et al*. (2018) for reported studies of wastewater treatment systems for parasites reduction efficiency in Africa revealed that WWTPs had higher reduction efficiency. The analysis indicated that parasitic helminths removal from wastewater by WWTPs had a mean reduction of 98.9% followed by constructed wetland 97.4% and activated sludge with 93.3%. However, for most reported studies in Africa only constructed wetlands systems were consistently met WHO helminths eggs standards of ≤1 egg per litre for wastewater to be reused for unrestricted agriculture irrigation (WHO, 2006).

Nevertheless, it has been observed that reduction efficiency of parasitic helminths was higher compared to protozoa for most of reported studies in Africa. It has been proposed to be caused by various factors including; higher concentration of protozoa cyst in inlets wastewater and their smaller size which impact to lower settling velocity compared to parasitic helminths eggs (Konaté *et al*., 2013; Jemli *et al*., 2015). Nevertheless, contamination of recovered wastewater with intestinal parasitic helminths such as STH and some cestodes like *T. solium* are more threatening risk to public health compared to protozoa. This is due to fact that eggs of these parasitic helminths are more environmentally resistant due to presence of strong outer layer(s) which facilitate their long survival and viability. In addition, intestinal parasitic helminths have low infective dose required to cause infection to a new host compared to protozoa (Gaasenbeek and Borgsteede, 1998; Jimenez, 2007).

2.1.4 Intestinal parasitic helminthes

Helminths (worms) are eukaryotic multicellular organisms. The most common parasitic helminths which inhabit the human gut are; nematodes (roundworms), cestodes (tapeworms) and trematodes (flatworms). With exception of *Strongyloides sp*. usually parasitic helminths cannot multiply within the host. Among intestinal parasitic helminths, four species known as STH *A. lumbricoides* (roundworm), *T. trichiuria* (whipworm), *A. duodenale* and *N. americanicus* (hookworms) are most commonly reported in developing countries (Cappello, 2004; Siza *et al*., 2015). High prevalence of these infection is mostly associated with poor sanitation facilities and lack of adequate water (Karan *et al.*, 2012).

Savioli and Albonico (2004) estimated that more than 1.5 billion people globally were infected by *A. lumbricoides* whereas, 1.3 billion were infected by hookworms and one billion peoples were infected by *T. trichiura.* Additionally, infections by intestinal parasitic helminths lead into multiple health problems with a long term effect such as growth retardation, reduced mental development, malnutrition, and increase in susceptibility to other infections (Liu *et al*., 2015; Siza *et al*., 2015). Children are most susceptible group which is in high risk of infection by intestinal parasitic helminths. Though immune compromised groups of people such as people with Human Immunodeficiency Virus (HIV) or Acquired Immunodeficiency Syndrome (AIDS) infection and pregnant women are also at risk and susceptible to complication caused by intestinal parasitic helminthes (Embrey, 2003).

Nevertheless, other species of intestinal helminths are not widely prevalent. However, in endemic settings a particular species of intestinal parasitic helminths can be more dangerous to threaten economy and public health of community around. For instance, *T.solium* is the among the most dangerous intestinal parasitic helminths which has significant economic and public health impact in most developing countries like Tanzania. It is zoonotic parasitic helminths responsible for porcine cysticercosis and human Taeniasis/cysticercosis that may lead into neurocysticercosis which is commonly associated with adaptive epilepsy, syncope, paralysis and chronic headache in human (Ngowi *et al*., 2008; Coral *et al*., 2014; Kimbi and Lekule, 2016; Gabriël *et al*., 2017; Jha *et al*., 2017). It is estimated that more than 2.8 million disability adjusted life years (DALYs) lost and about 300 000 peoples were reported to be infected with *T. solium* globally in between 2010 and 2015 (Torgerson *et al*., 2015).

2.1.5 Assessment and analytical techniques of parasitic helminths from wastewater and irrigated crops

For suitable exploitation of full potentials of reused wastewater for crop irrigation there should be proper management and close monitoring strategies to ensure sustainability and safeguard public health. Quantification and identification of biological infectious agents is the most common monitoring strategies used according to FAO and WHO standard guidelines (FAO, 2002; WHO, 2006). Conventional traditional methods based on sedimentation and/or flotation are the most common analytical procedures used for quantifying parasitic helminths eggs in wastewater, sludge and excreta which basically relies on their visual identification and enumeration. Few to mention are; modified Bailenger Method (MBM), United States Environment Protection Agency (US EPA) and Arther Fitzgerald. In fact, all these analytical procedures have two principal steps in common; the separation of as many eggs as possible from other particles in the sample and microscopic identification of concentrated sediments (pellets) in the mixture of many other particles. This implicate that conventional parasitological identification requires proper trained technician who is able to discriminate parasitic helminths eggs from other particles.

Majority of studies done to assess and identify parasitic helminths eggs in wastewater, sludge and irrigated crops in Africa used MBM as analytical technique *(Hajjami et al*., 2013; Nwele *et al*., 2013; Sabbahi *et al*., 2018). Modified Bailenger method (Ayres and Mara, 1996) is based on floatation procedures using zinc sulphate with 1.8 specific gravity as floating solution. It is widely used since it is comparatively simple, spent short time to complete and has broad range of helminth eggs identification. But it is not appropriate for identification of operculated eggs such as those of *Fasciola sp*. *Clonorchis sinensis* and *Diphyllobothrium latum* (Ayres and Mara, 1996). On the other side, the use of floating solution with 1.8 specific gravity limits the method to be used for identification of high density eggs such as Taeniid eggs which has about 3.0 specific gravity.

United States Environment Protection Agency analytical technique has reported to be used by few studies in Africa. Compared to MBM, the US EPA is more complex also use more time to complete proper assessment and identification of parasites in the collected samples and also the whole sample are fully used in the identification which limit sub-processing of the sample to enhance replication of results (Konaté *et al*., 2013).

Generally, conventional tradition methods can identify parasites up to genus level but fail to differentiate them to the species level and hence limit for the species specific intervention control of infection in most endemic settings. The advert of new methods for quantification of parasitic helminths eggs are considered to be the more complex way of dealing with environmental samples. The use of image processing algorithms (IPA) to identify and quantify parasitic helminth eggs from photographs taken using a microscope is among the most efficient technique of new era (Jiménez *et al*., 2017). This method is based on selected characteristics of helminth eggs that allow their correct identification without the need for a highly trained technician and it proved to have high specificity (capacity to discriminate between helminth eggs and other debris) and sensitivity (capacity to correctly identify and classify the different species of helminth eggs).

The advent of molecular techniques accompanied with genomic sequencing are remarkably increased the feasibility of developing polymerase chain reaction (PCR)-based methods as diagnostic tools for helminth parasites. Definite gene sequences of parasitic helminth eggs can be detected with PCR, quantitative PCR (qPCR) and other nucleic acid based methods from small quantities of samples. These techniques can also identify parasitic helminth eggs to species level (Gordon *et al*., 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Mbeya city and Mbeya rural district in Mbeya region for five months from December 2018 to April 2019. The study sites are located in Southern highlands of Tanzania, between latitudes 7 and 9 °S and longitudes 32 and 35 °E (Fig. 1). The climate of the region is subtropical and it receives abundant and reliable rainfall. Rainfall in most parts of the region including Mbeya city and Mbeya district have mono-modal rainfall pattern. The dry season often starts in June and ends in December whereas the wet season begins in January and ends May. Annual rainfall may range from 650 mm to more than 2600 mm. Mbeya region lies at an altitude range from 500 to 2400 meters above sea level with temperatures range between 16 °C in the highlands and 25 °C in the lowland areas (URT, 2007).

In 2012, human population was estimated to be 385,279 and 305,319 in Mbeya city and Mbeya rural district, respectively (NBS, 2013). About 10% of agricultural practice in the region is through irrigation of which large part of land in peri-urban and city adjacent wards of Mbeya rural district use channelled recovered wastewater from Kalobe wastewater treatment ponds for crop irrigations. Additionally, both Mbeya city and Mbeya rural district are famous for pig production and marketing, whereby, smallholder pig production are mostly practiced especially in peri-urban areas of Mbeya city and rural areas of Mbeya district. Thus, both study sites have been purposively selected due to their illustrious characteristics of reused wastewater irrigated vegetable productions and high production of pigs and pig marketing. All these facilitate transmission dynamics of various intestinal parasitic helminths such as STH and also the place is associated with high prevalence of *T. solium* taeniasis/ cysticercosis in pigs and human beings (Wilson and Swai, 2014).

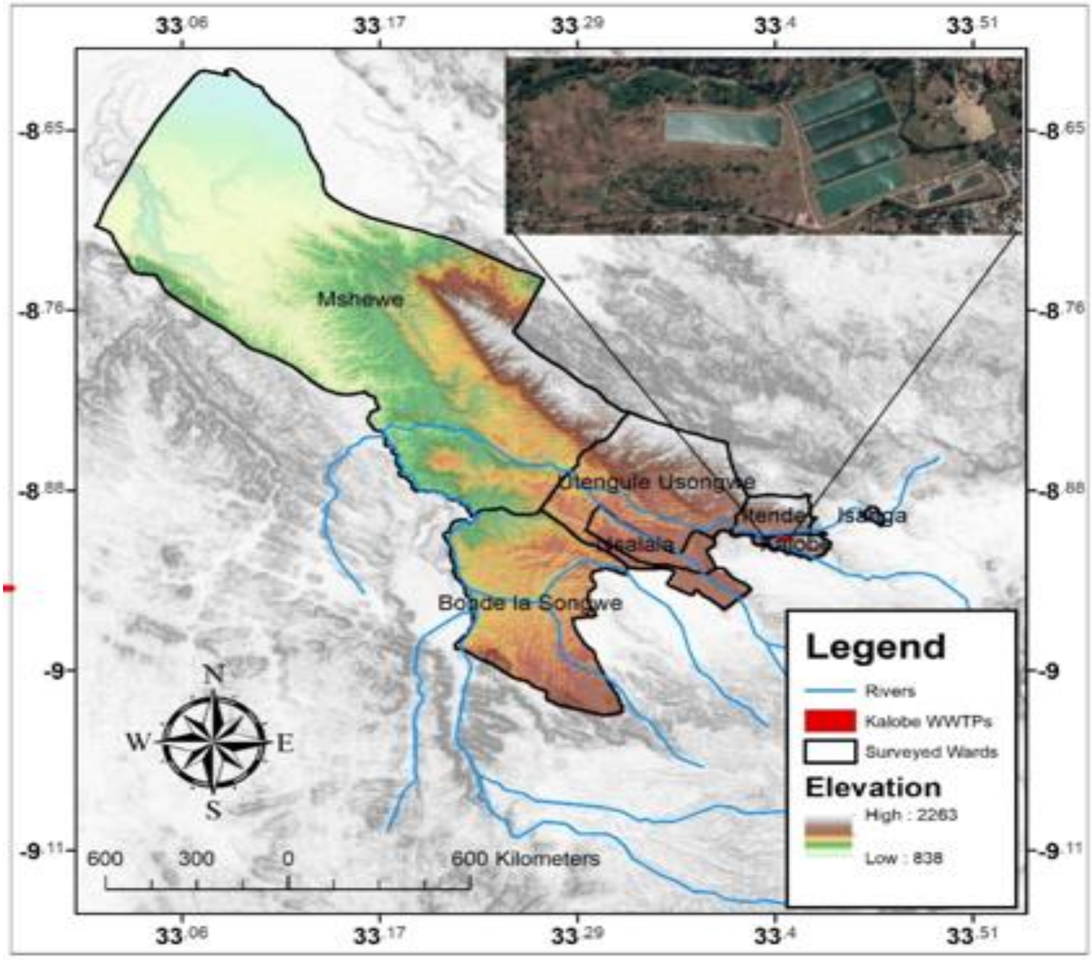

Figure 1: Map of Mbeya city and Mbeya district showing study sites. The study was conducted in six wards demarcated by solid dark lines. The satellite image shows Kalobe wastewater treatment ponds.

3.2 Study Design

This study adopted cross-sectional study design. In one part of the study the convenient exploratory method was used to assess vegetable farmers' perceptions and managerial practices regarding to reused wastewater for irrigation and consumption of wastewater irrigated vegetables in relation to transmission of parasitic helminths.

Concurrently, in the second part of the study the purposive sampling method was used in sampling effluent water from Kalobe wastewater treatment ponds (WWTP), polluted streams channeled with wastewater (PSCW) and vegetables irrigated by reclaimed wastewater.

3.3 Samples Collection

3.3.1 Assessment of farmers' managerial and vegetable consumption practices, and perception

Smallholder vegetable farmers were purposively selected and interviewed using a semi-structured questionnaire. The participants came from purposively selected nine (9) villages of four (4) wards which are involved in reused wastewater crop irrigation in Mbeya rural district and three (3) streets of peri-urban in Mbeya city. The questionnaire was designed to address demographic variables (including respondents' sex, age, level of education, occupation, marital status and household size), type of vegetables cultivated, access to water resources for irrigation, vegetable consumption regime, knowledge and perceptions toward health risks related to reuse of wastewater and its irrigated vegetables consumption (Appendix 1). The aim was to assess awareness and perceptions of smallholder vegetable farmers on contamination risks of consumption of crops irrigated by recovered wastewater. Rapid assessment checklist also was used to assess water quality and irrigation practices (Appendix 2). For the sake of analysis of perception and awareness, the numerical values "1" for Yes and "2" for No in binary questions were used whereas for open ended question the codes were 1= "easily to access and high fertile", 2= "Not much good due to suspect of diseases", 3= "Not good because it has chemicals", 4= "Easily to access and with high fertility but contain chemicals which may kill some crops", 5= "Easily to access and high fertile but may have disease pathogens".

3.3.2 Wastewater sample collection

Effluent wastewater from Kalobe WWTPs were collected twice a week, whereas polluted water from PSCW and CPW were collected at least once a month from each respective ward of surveyed area for the whole period of study. A grab sample of 10 L was collected from each respective wastewater source. The samples were then allowed to settle for sedimentation for 24 hours at Mbeya College of Health and Allied Sciences (MCHAS) laboratory, and about 1 L of recovered sediments were stored in normal room temperature for further processing.

3.3.3 Vegetables samples collection

Vegetable samples which were directly irrigated by reclaimed wastewater or polluted water were collected according to availability of such crops from farmland or gardens alongside wastewater channels in rural Mbeya district and peri-urban Mbeya city. The samples were then packed in sterile plastic bags and taken to MCHAS laboratory. Within a same day about 200 g of collected vegetable were thoroughly washed by tap water in 10 L plastic gallon, then water used were allowed to sediment for about 24 hours. About 1 L of recovered sediment was stored in normal room temperature for further processing.

3.4 Recovery and identification of intestinal helminths eggs from samples

Parasitological identification process for both stored sample from wastewater and vegetables were performed at Mbeya Zonal Referral Hospital Parasitology Laboratory within four (4) days after sample collection.

Modified Bailenger method (MBM) (Ayres and Mara, 1996) were adopted for intestinal helminths eggs identification. Recovered sedimented water from wastewater and vegetables which were stored in the sterilized collecting plastic bottles were transferred to

50 mL centrifuge tubes. All the sediments were centrifuged at 40 000 rpm for 15 min and the supernatant were carefully removed without disturbing the pellets. The pellets were transferred into one tube and thorough rinsed with Triton X-100 detergent to ensure removal of all pellets. Then material were recentrifuged again at 40 000 rpm for 15 min. Floatation technique was used by suspending the pellets in equal volume of aceto acetic buffer, pH 4.5 for concentration of helminths eggs, followed by addition of ethyl acetate and centrifugation at 40 000 rpm for 15min. Thereafter, the pellets obtained were re-suspended using zinc sulphate solution ($ZnSO_4$) with 1.3 specific gravity. Two McMaster slides were used for microscopic identification and counting of selected helminths eggs and the average number of eggs per two slides were recorded. Identification were based on eggs' morphological characteristics and size. Then quickly the eggs from microscopic slide were mixed with remaining sample, washed and suspended using distilled water and recentrifuged at 40 000 rpm for 15 min, then pellets obtained were stored at -20 ^{0}C ready for DNA isolation.

3.5 Isolation of Genomic DNA

Prior to DNA extraction the eggs in egg-containing suspension were subjected to pre-lysis stage. Taeniid eggs frozen at -20°C were thawed at room temperature and centrifuged at 2,500 *g*. Approximately 50 µL of the egg-containing suspension were mixed with 25 µL 1M potassium hydroxide (KOH) and 7 µL of 1M dithiothreitol (DTT) and incubated for 15 min at 65°C for lysing the eggs.

Alkaline lysis was neutralized by adding 60 µL 2M Tris-HCl (pH 8.3) and 5 µL 10 M concentrated hydrochloric acid (HCl) (Dyachenko *et al*., 2008). Afterwards, genomic DNA were extracted from eggs by using a DNeasy tissue kit (QIAGEN, Hilden, Germany) following protocol procedures recommended by the manufacturer. DNA concentration and purity (quantification) were then assessed by measuring absorbance at 260 and 280 nM using a spectrophotometer (BIOCHROM LTD, Cambridge, England).

3.6 PCR assay for Taenia solium Species Identification

Detection of *T. solium* DNA were performed using multiplex polymerase chain reaction (PCR) assay targeting the cytochrome c oxidase subunit 1 gene (COX-1) of mitochondrial DNA (mDNA) (Yamasaki *et al*., 2004). Based on the nucleotide sequences of COX-1 from human *T. solium*, the following primers were adopted to amplify different sizes of products (Table 1) (Yamasaki *et al*., 2004).

Table 1: Primers for multiplex identification of *T. solium*

Species	**Type of primer**	**Nucleotide bases chain (5'→ 3')**	**Expected band size (bp)**
T. solium American/African genotype (Tsol/Amer),	Forward primer	GGTAGATTTTTTAATGTTTTCTTTA	720
T. solium Asian genotype (Tsol/Asia)	Forward primer	TTGTTATAAATTTTTGATTACTAAC	984
Universal for all *T. solium* species	Reverse primer	GACAT AACATAATGAAAATG	

A PCR cocktail contained mixed primers and 0.5 U of the *Ex Taq* DNA polymerase Hot Start (TaKaRa, Tokyo, Japan) in 25 µL of a reaction mixture. Standard multiplex PCR protocols consisted at initial denaturation of 94 °C for 10 min, followed by 40 cycles of denaturation (30 sec. at 94 °C), annealing (30 sec. at 56 °C) and extension (90 sec. at 72 °C), plus one cycle of 5 min. at 72 °C.

3.7 Visualization of PCR Products and Interpretation of Results

The amplification of specific multiplex PCR product was checked by gel electrophoresis in 1.0% agarose gel stained with 4 µL of gel red stain. 100 bp ladder was used for band size visualization. The band size of 720 bp implies that the sample is positive for *T. solium* eggs of Africa or South American genotype, whereas, the band size of 984 bp indicate that the sample is positive for *T. solium* eggs of Asia genotype.

3.8 Statistical Analysis

Data from the questionnaire were entered into SPSS (version 23, IBM) database. Statistical analysis was done after testing the assumption of normality. Descriptive statistics (frequency, mean and Standard deviation) were performed using SPSS (version 23, IBM). For sociological data, Multivariate analysis of covariance (MANCOVA) was used to examine the difference in perception and awareness between gender, age, marital status, size of households, time involved in crop production and educational level with selected farming and vegetable consumption practices. For parasitic helminths eggs identification, student t-test and chi square was performed to determine the statistical difference between the concentrations of parasite eggs in origin of samples collected. Statistically significant difference was assumed at the 5% level.

CHAPTER FOUR

4.0 RESULTS

4.1 Managerial Practices, Awareness and Perceptions of Smallholder Farmers Regarding Consumption of Reused Wastewater Irrigated Vegetables

4.1.1 Field observation

Direct observation were conducted in peri-urban Mbeya city and Mbeya rural district provided baseline information on water quality and irrigation practices of smallholder vegetable farmers. The community of peri-urban Mbeya city and Mbeya rural district practiced mixed farming system with loosely established irrigation systems. Out of 61 surveyed vegetable fields, 37.7% of vegetable farmers pumped wastewater from main stream using pipes and sprinklers and 44.3% fetched wastewater from main streams using hand eureka cans while only 18% used gravity through channels from the main streams for irrigation of vegetables. The main vegetable crops cultivated were the mixture of folded (Chinese, lettuce, kale, cabbage and spinach) and unfolded (amaranthus and night shade). Also fruits and roots type of vegetables were commonly grown such as tomatoes, okra, eggplant, green peppers, green tomatoes and carrots. Irrigation water channeled to the fields had moderate speed with vast amount of particles and suspensions. Rotation crops cultivation were practiced in the area whereby cereals (mostly maize) and legumes (such as beans and peas) were cultivated during rain seasons whereas vegetable crops were cultivated during dry seasons through reused wastewater irrigation.

4.1.2 Demographic profile

A total of 61(39 males and 22 females) smallholder vegetable farmers participated in the survey. Male farmers had higher years of formal education (41% reached secondary to higher education) compared to female (31% reached secondary to higher education).

However, the difference was not statistically significant (P>0.05). About 44.3% of the farmers aged between 41-60 years whereas 37.7% and 18% aged between 18-40 years and above 60years, respectively. Majority of farmers (67.2%) seems to have between one to five years of experience in vegetable crops production using recovered wastewater in the study area and about 31.1% have experience of more than 5 years (Table 2). Results from analysis of variance (ANOVA) of demographic factors (gender, age, marital status, number of households, education level and time involved in crop production) of farmers participated showed that, there were no statistically significant difference between wards surveyed during study period (p>0.05).

Vegetable farmers from all wards surveyed required permission to use recovered wastewater of the main channels from local authority. Farmers from Utengule usongwe ward require permission from local farmers union authority ("Umoja wa wakulima") while all other wards require permission from local village or street authority. The results of the survey revealed that about 59 (96.7%) of vegetable farmers participated in the survey uses vegetables they produce for home consumption and for sell whereas only 2(3.3%) produce vegetables only for sell.

Table 2: Demographic profile of smallholder vegetable farmers in peri-urban Mbeya city and Mbeya district

	Wards							Total
	UTU	NSL	BLS	MSH	NSG	KLB	ITD	
Gender								
Male (n)	17	2	7	5	5	2	1	39
Female (n)	13	2	3	2	2	0	0	22
Age								
18-40 years (n)	14	1	5	1	2	0	0	23
41-60 years (n)	12	3	4	3	3	2	0	27
Above 60 years (n)	4	0	1	3	2	0	1	11
Education level								
No formal education (n)	1	0	0	1	0	0	0	2
Primary education (n)	18	2	6	4	5	0	1	36
Secondary education (n)	8	0	4	0	2	1	0	15
Higher education (n)	3	2	0	2	0	1	0	8
Time involved in crop production								
1-5 years (n)	21	4	7	2	5	2	0	41
Over 5 years (n)	5	0	3	5	2	0	1	16

n= Number of participants

4.1.3 Awareness and perception of risk of contamination vegetables irrigated by reused wastewater

Different ideas were noted regarding awareness and the way smallholder vegetable farmers perceived about risk of using recovered wastewater from WWTPs. Majority of farmers (55.8%) were aware on disease pathogens' contamination risks of reused wastewater for crop irrigations and few perceived to be not good due to chemicals contamination which killed crops (Fig. 2).

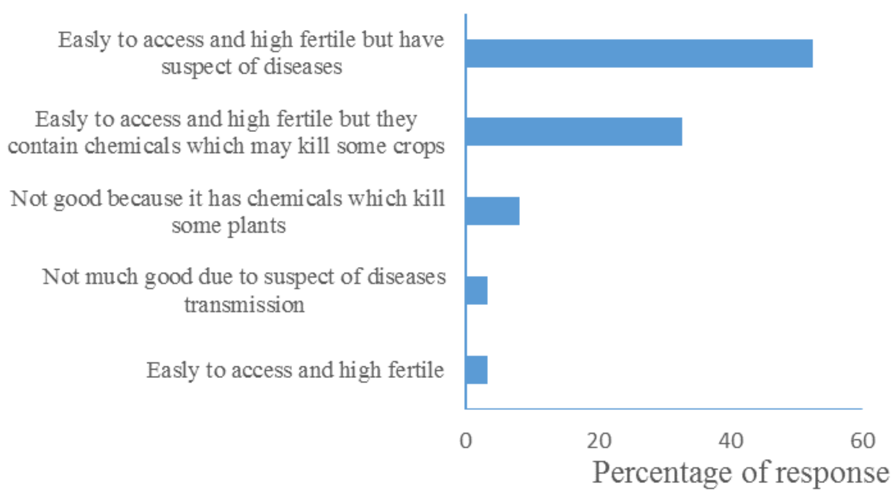

Figure 2: Awareness of smallholder farmers on risk of contamination of reused wastewater for crops irrigation

Perceptions and awareness towards enteric disease pathogens contamination of vegetables by reused wastewater irrigation were assessed through the responses of 4 binary (yes or no) type questions and one open ended question. MANCOVA were done to highlight results of variation in responses by gender, age, education level and time involved in crop production as associated with perception and awareness of participants (Table 3).

Table 3: Multivariate analysis of covariance of selected farming and consumption features as functions of demographic variables

Variables	Coeff.	value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Wilks' Λ	0.452	14.830[b]	4.000	49.000	0.000	0.548
Participant age	Wilks' Λ	0.849	2.185[b]	4.000	49.000	0.084	0.151
Education level	Wilks' Λ	0.489	3.364	12.000	129.933	0.000	0.212
Time involved in crop production	Wilks' Λ	0.809	2.894[b]	4.000	49.000	0.031	0.191
Participant sex	Wilks' Λ	0.937	0.817[b]	4.000	49.000	0.520	0.063

b. Exact statistic

A separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alpha level of 0.025. There was significant difference between participants' education level on awareness of health risks and perception of negative health problems associated with consumption of vegetables irrigated with reused wastewater, $F(3,52)=12.769$, $p=0.000002$, $\eta^2=0.424$ and $F(3,52)=3.873$, $P=0.014$, $\eta^2=0.183$, respectively. On the other hand, ANOVA showed significant difference between participants' time involved in vegetables production on awareness of any health problem associated to reused wastewater for vegetables irrigation, $F(1,52)=9.36$, $P=0.004$, $\eta^2=0.153$.

During survey, about 58 (95%) participants were aware of health problems associated with reused wastewater for vegetables irrigation. Amongst them, 49 (80%) mentioned more than one health problem (such as, stomach problem, diarrhea diseases, helminthic diseases, swelling of legs and skin rushes) (Fig. 3). However, when were asked on their own experience on health problems from reused wastewater for vegetable irrigation 51(83.6%) of vegetable farmers participated in the survey mentioned to have succumbed to health problems such as diarrhea and other stomach diseases associated to consumption of vegetables irrigated by reused wastewater. Additionally, about 45 (73.8%) of respondents indicated that, children were at high risk of these health problems compared to adults.

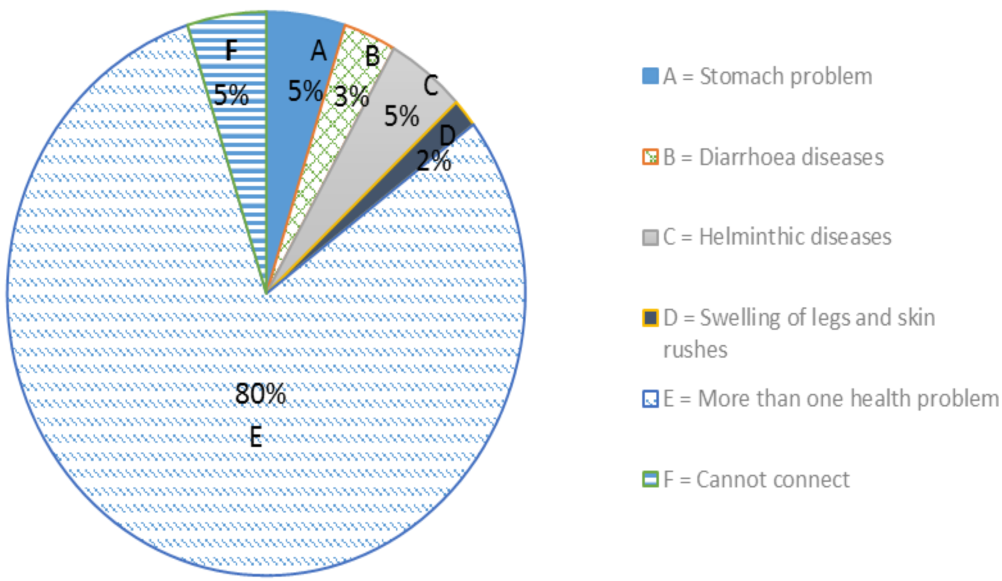

Figure 3: Vegetable farmers' percentage of awareness in enteric health related problem. A-F are responses from participants

When the respondents were asked to explain on why they associate diarrhea cases with consumption of vegetables irrigated with reused wastewater, a good number of the respondents 25 (41%) mentioned the increase of cases during vegetable harvesting periods. Interestingly, about 38% of respondents failed to explain the connection between diarrhea cases with vegetable consumptions (Fig. 4).

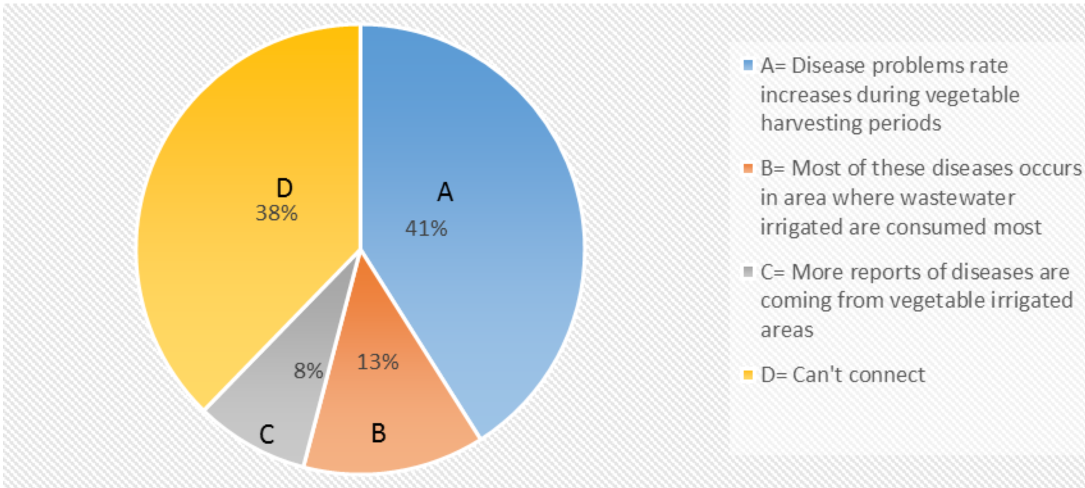

Figure 4: Smallholder vegetable farmers' awareness on association of enteric diseases with reused wastewater and consumptions of vegetables irrigated with reused wastewater. A-D are responses from participants

4.1.4 Vegetables farming practices

During the study period the results revealed that, majority of farmers participated in the survey 39 (63.9%) use crop production as their main income generating activity (MIGA) whereas, 19 (31.1%) and only 3 (4.9%) use formal employment and livestock production as their MIGA, respectively. However, apart from MIGA most of those farmers had other income generating activities such as (crop production and livestock production, crop production and formal employment, crop production and causal labour or crop production, livestock production and other activity). When the respondents were asked on what do they do with vegetables leftovers after harvesting, about half 32 (52.5%) mentioned to use them for feeding livestock at home, while 29 (47.5%) of them said that they just destroy them in fields after harvest. Interestingly, all of vegetable farmers' participated in the survey reported to use the same irrigation water for vegetables post-harvest washing of crops in the field.

4.1.5 Vegetables consumption practices

Vegetable consumption practices among smallholder vegetable farmers were assessed through responses of two multiple responses questions, two open response questions and 2 binary (“Yes” or “No”) type questions. The results showed that, majority of the respondents (88.5%) have more than one source where they can obtain vegetables for home consumption, such as their own farm fields, buy from local markets and vendors in the streets or villages. In addition, for those who can buy vegetables from local markets or vendors, about 98.4% normally do not asks about the source of water for irrigation of those vegetables. However, vegetable consumption was mentioned to be regularly taken in daily meals especially during vegetable harvesting seasons. Consumption of raw vegetables (salad and raw carrots) was mentioned to be normal practice by the majority (80.3%) of the respondents (Fig. 5). Although, when asked the question on the normal practice of cleaning vegetables at home, 100% of all vegetable farmers participated in the study uses normal clean water to wash vegetables.

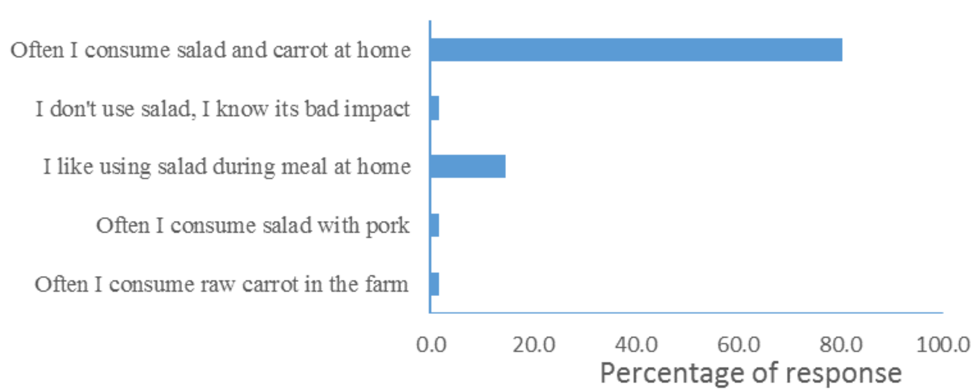

Figure 5: Distribution of vegetable farmers’ raw vegetables consumption practices responses

Association of vegetable consumption practices with participants', gender, age, education level, and time involved in vegetable crop production were analysed through multivariate linear regression model. The results highlighted that, education level influenced vegetable consumption practices ($P<0.05$) among smallholder vegetable farmers who participated in the survey (Table 4).

Table 4: Multivariate linear regression of consumption needs and practices as function of selected demographic variables

Variables	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	Coeff. B	Std. Error	Beta		
Consumption needs and practices					
(Constant)	1.027	.404		2.544	.014
Participant sex	-.056	.109	-.058	-.515	.609
Participant age	-.020	.078	-.031	-.259	.796
Education level of participant	.329	.075	.539	4.397	.000
Time involved in crop production	-.030	.127	-.031	-.232	.817

4.2 Parasitological identification of selected helminths eggs from field samples

To assess the risks of pathogenic parasitic helminths transmission associated with reused treated wastewater for agriculture irrigation. Effluent waste water from Kalobe wastewater treatment ponds, downstream polluted streams channeled with effluent water of Kalobe WWTPs, crops taken from fields alongside polluted streams channeled by effluent water from Kalobe WWTPs of which the water were used for irrigation, and upstream of polluted stream before joining with effluent water from Kalobe WWTPs were analysed. A total of 44 wastewater samples were collected, of which 25 samples were from Kalobe WWTP, 14 were from polluted streams channeled with wastewater (PSCW) in Mbeya district villages and 5 were samples from peri-urban streams in Mbeya city.

4.2.1 Qualitative identification of parasitic helminths eggs load from water samples

Parasitological identification of helminths eggs from effluent of WWTPs, polluted streams channeled by treated wastewater (PSCW) and upstream of polluted stream before it join with treated wastewater (CPW) allowed the identification of the load and set of parasitic helminths eggs potentially pathogenic for humans. The eggs obtained belong to various groups of parasitic helminthes: Nematodes, Cestodes and Trematodes (Table 5).

Table 5: Parasitic helminths eggs obtained from effluent wastewater, polluted water and vegetables

Helminths		**Dec. 2018**			**Jan. 2019**			**Feb. 2019**			**Mar. 2019**			**April. 2019**		
		A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Nematodes	*Ascaris sp.*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	Trichuris sp.	+	-	-	+	+	-	+	+	-	+	-	-	+	-	-
	Hookworm spp.	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
	Enterobius sp.	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-
	Strongyloides sp.	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-
Cestodes	*Taenia sp.*	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+
	Hymenolepsis sp.	+	+	+	+	+	-	+	+	-	+	-	-	+	+	-

A: Effluent wastewater, B: Polluted stream water channeled with treated wastewater, C: Vegetable samples,

(+): Present, (-): Absent

Throughout the study period, parasitological assessment revealed the presence of at least one parasitic helminth eggs in 88% (n=25) samples of WWTPs, 78.6% (n=14) sample of PSCW and in 75% (n=12) samples of vegetables Collected (Fig. 5).

Table 6: Percentage distribution of different types of parasitic helminth eggs in positive samples

Helminths	WWTP	Polluted stream	Vegetables
Ascaris sp.	84% (21/25)	78.6% (11/14)	85.7% (6/7)
Trichuris sp.	28% (7/25)	21.4% (3/14)	28.6% (2/7)
Hookworm sp.	72% (18/25)	57.1% (8/14)	71.4% (5/7)
Strongyloides sp.	52% (13/25)	35.7% (5/14)	71.4% (5/7)
Enterobius sp.	40% (10/25)	14.3% (2/14)	14.3% (1/7)
Taenia sp.	64% (16/25)	57.1% (8/14)	57.1% (4/7)
Hymenolepsis sp.	44% (11/25)	42.9% (6/14)	42.9% (3/7)

Ascaris sp. eggs had a highest average recovery rate from both effluent WWTP and PSCW being 84% and 78.6%, respectively, whereas, *Trichuris sp*. eggs found to be the least being 28% and 21.4%, respectively, during the whole study period (Table 6). Also it should be noted that during December, 2018 and January, 2019 sampling period, the trend of recovery of eggs from vegetables irrigated by reused wastewater *Ascaris sp*. eggs was still high (85.7%) whereas *Trichuris sp*. eggs showed the least.

4.2.2 Quantitative identification of parasitic helminths eggs load from water samples

Quantitative assessment of intestinal helminths eggs in effluent of WWTP, PSCW and CPW indicated that, overall mean contamination with helminths eggs was 1.143 egg/L, 0.667 egg/L and 0.064 egg/L, respectively. Considering overall mean variation of intestinal parasitic helminths eggs, the trend decreased from WWTP to PSCW, while it was almost zero in CPW (Table 7).

Table 7: Overall monthly mean number of eggs per litre of water of sampled sources

	Mean number of eggs/litre of water				
Sample source	Dec. 2018	Jan. 2019	Feb. 2019	Mar. 2019	Apr. 2019
WWTP	1.31	1.204	1.036	1.071	1.095
PSCW	0.821	0.823	0.643	0.429	0.619
CPW	0.119	0.2	0	0	0

4.2.2.1 Monthly variation of parasitic intestinal nematode eggs

During the five months of study period it has been noted that, monthly analysis in the load of parasitic intestinal nematodes showed *Ascaris* eggs had higher load of eggs recovery (mean 1.93eggs/L and 1.60eggs/L) followed by hookworm eggs (mean 1.60eggs/L and 0.79eggs/L) whereas *Trichuris sp*. eggs showed the least (mean 0.48eggs/L and 0.19eggs/L) in eggs load recovery in effluent of WWTPs and PSCWs, respectively. The trend showed that, parasitic helminth eggs concentration tend to decrease as they move from effluent of WWTP to downstream of PSCW where they were directly used for irrigation of vegetable fields. Additionally, analysis showed that no parasitic intestinal nematode identified from CPW in February, March and April, 2019. However, 0.33 eggs/L of *Enterobius sp*. were recorded in December, 2018 and January, 2019 whereas only 0.5 eggs/L of *Strongyloides sp*. were recorded in Dec. 2018 (Fig. 6). Further analysis revealed that there was no statistically significant difference

(P>0.05) in eggs concentration between effluent water of WWTPs and PSCWs during the whole time of the study.

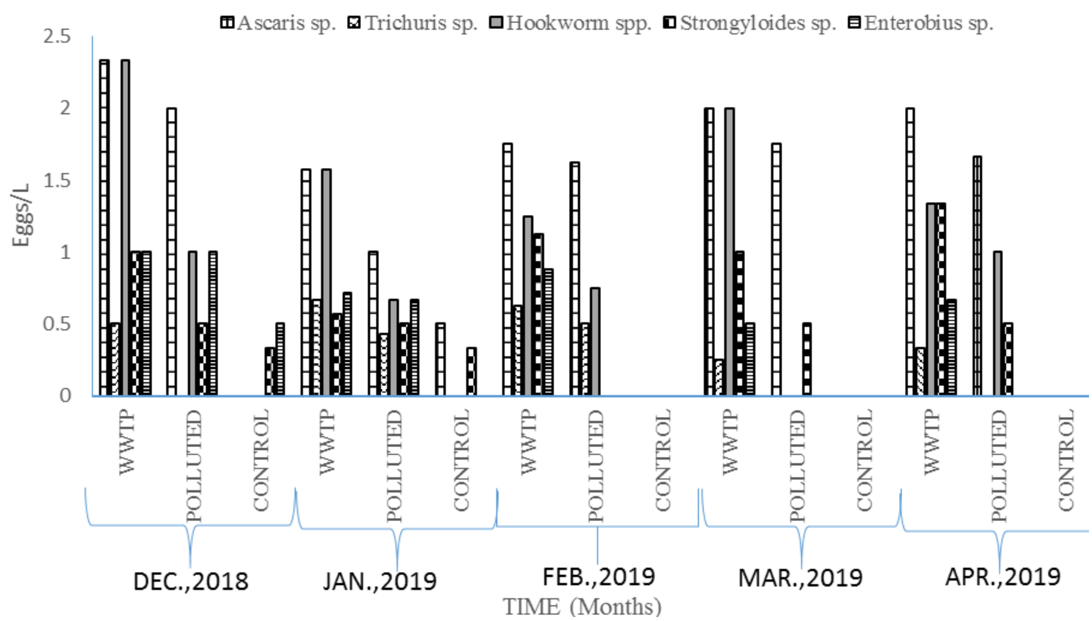

Figure 6: Mean monthly load distribution eggs/L for parasitic intestinal nematodes for five months of study

4.2.2.2 Monthly variation of parasitic intestinal cestode eggs

Two selected species of parasitic intestinal cestodes (*Taenia spp*. and *Hymenolepsis spp*.) were identified in parasitological examination during the time of the study. It was noted that, mean concentration of Taeniid eggs were slightly higher (1.23eggs/L and 0.85eggs/L) in all five months of the study compared to *Hymenolepsis* eggs mean concentration (1.03eggs/L and 0.48eggs/L) in effluent of WWTPs and PSCWs, respectively (Fig. 6). With exception to January 2019 none of the two cestode species has been detected in CPW. The trend revealed that, there were slightly decrease in cestode eggs concentration as water moves from PSCW to CPW (Fig. 7).

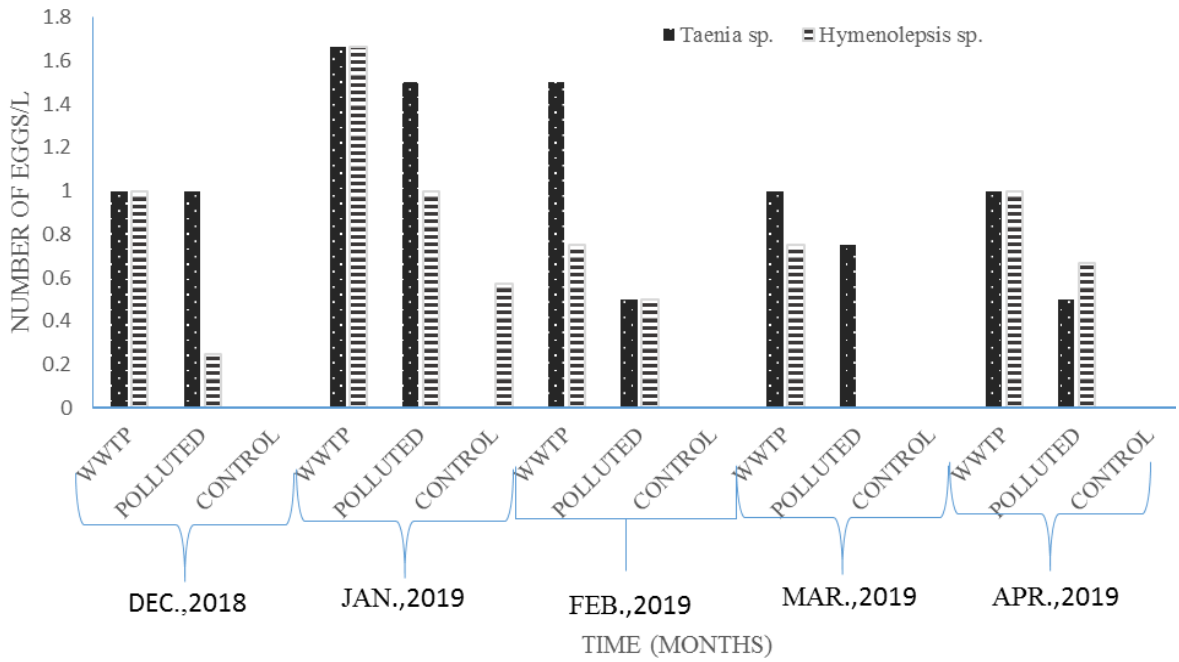

Figure 7: Mean monthly load distribution eggs/L for parasitic intestinal cestodes for five months of study

4.2.3 Quantitative identification of parasitic helminths eggs load from vegetable samples

Quantitative assessment of intestinal parasitic helminths eggs in vegetable samples showed that, mean eggs concentration was about 3.3 eggs/100g for December, 2018 and January, 2019. However, the drastic drop in mean concentration were observed to be 0.8 eggs/100g, 0.5 eggs/100g, and 0.3 eggs/100g in February, March and April, 2019, respectively (Table 8).

Table 8: Mean parasitic intestinal helminthes eggs distribution from vegetable samples collected

		Helminths eggs from vegetables (Eggs/100g)				
		Dec. 2018	Jan. 2019	Feb. 2019	Mar. 2019	April. 2019
Nematodes	*Ascaris sp.*	6	3.75	1.875	3.75	0
	Trichuris sp.	2.25	0	0	0	0
	Hookworm spp.	3	5.625	3.75	0	0
	Enterobius sp.	1.5	1.875	0	0	0
	Strongyloides sp.	3	5.625	0	0	0
Cestodes	*Taenia sp.*	3.75	5.625	0	0	1.875
	Hymenolepsis sp.	3.75	0	0	0	0

4.3 Molecular Detection of *Taenia solium*

Out of 56 samples of WWTPs, polluted water and vegetables collected, 30 samples were positive for Taeniid eggs in parasitological examination using light microscope (16 WWTPs, 9 polluted water and 5 vegetables). PCR analyses of 30 positive Taeniid eggs isolates showed that, 15 (26.8%) isolates were DNA positive for *T. solium*. Among the isolates identified representing 7(12.5%) WWTPs samples, 5 (8.9%) polluted water and 3 (5.4%) vegetable samples of positive Taeniid eggs samples detected through conventional parasitological identification (Table 9).

Table 9: *Taenia solium* DNA positive samples identified from Taeniid eggs isolates

Sample source	The ward where sample taken						
	UTU	BLS	NSL	MSH	NSG	KLB	ITD
WWTP	0	0	0	0	0	7	0
PSCW	3	0	0	1	0	0	1
Vegetables	2	0	0	1	0	0	0
Total	5	0	0	2	0	7	1

Polymerase chain reaction analysis of *T. solium* using COX-1 gene species specific primers resulted to 720 bp bands in 1% Agalose gel electrophoresis (Fig. 8). This denoted that, all the PCR positive *T. solium* were of American/Africa origin.

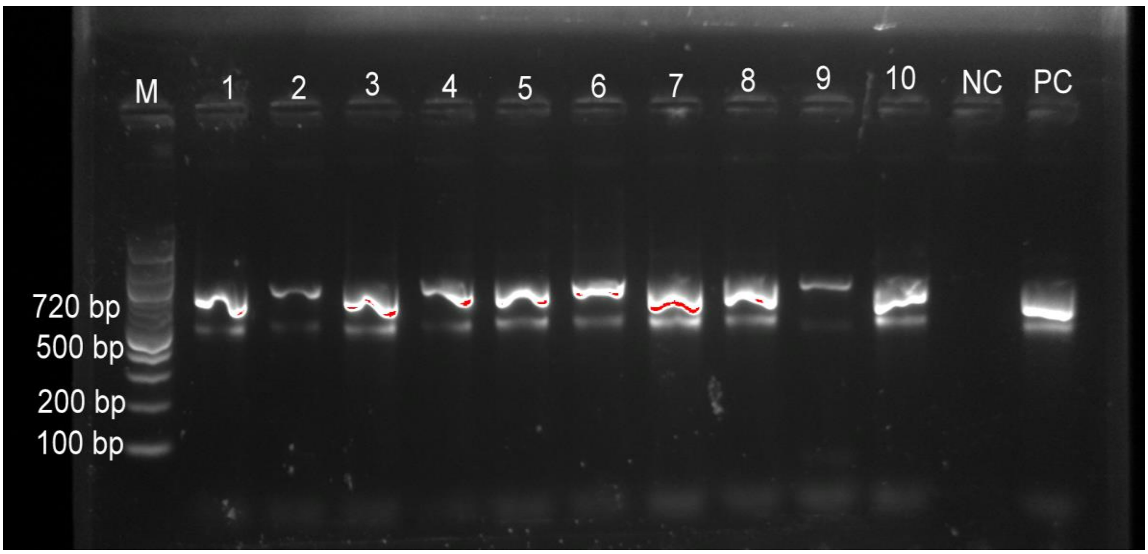

Figure 8: Agarose gel electrophoresis of *T. solium* based on amplification species-specific COX-1 gene sequences of mDNA positive PCR products. Lane 1-4 were Taeniid eggs isolate samples from Kalobe WWTPs, lane 5- 7 were Taeniid eggs isolates samples from PSCW, lane 8- 10 were Taeniid eggs isolate samples from vegetables, NC was negative control, PC was positive control and M was 100 bp ladder.

CHAPTER FIVE

5.0 DISCUSSION

This study demonstrated that reused wastewater for vegetable irrigation is a common way of crop production practiced by smallholder vegetable farmers in the study area probably due to its high nutrient value as noted by majority of the respondents (Fig. 2). Irrespective of the depth and level of understanding, most farmers were aware about possible health risk of reused wastewater for crop irrigation. More than half of the farmers (55%) were aware on disease pathogens' contamination risks of reused wastewater for crop irrigation (Fig. 3); however, majority of them lacked knowledge on association of enteric disease problems with irrigation using wastewater and consumption practices of irrigated crops like vegetables.

The results showed that, there were significant differences between participants' education level and time involved in vegetables production with respect to perception and awareness of risk of vegetables contamination with enteric disease pathogens by reused wastewater (Table 3). In fact, higher level educated respondents who were relatively few, doubted the quality of PSCW used for crops irrigation due to suspected disease pathogens transmission to human and chemical contamination for plant health. This imply that even though formal education prepares individuals to be more informed about the general contamination health risks of the environment, specific education particularly on connection of enteric diseases with reused wastewater for irrigation is needed. Additionally, formal education does not provide particular knowledge on proper irrigation and consumption practices which reduce contamination health risks of produced crops like vegetables. The observation is in agreement with results of a study conducted in Morogoro, Tanzania (Samson *et al*., 2017). In that study, the Government officials who were assumed to be more educated had negative opinions on use of reused wastewater for crops irrigation due to possible health risks.

Regardless of the aversions that discourage the practice of reused wastewater for vegetable irrigation, farmers were convinced that motivational factors do outweigh aversions. Main factors that drive smallholder vegetable farmers to use recovered wastewater for vegetable irrigation are easy accessibility and high fertility of such water which increase yield of crop productions (Fig. 2). It has been noted in this study that more than 95% of the farmers grow vegetables for the purpose of home consumption and for sell to increase their income. Similar influencing factors on use of wastewater for crops irrigation have been reported in other studies in Jordan and in Bangladesh which mentioned low running cost, high fertility and the easy accessibility (Carr *et al*., 2011; Keraita *et al*., 2012).

The reported health risks associated with reused wastewater in crop farming is infections with intestinal parasites and bacteria to both producers and produce consumers (Blumenthal and Peasey, 2002). In this study, more than 80% of vegetable farmers were aware of more than one health problem associated with reused wastewater for vegetable irrigation (Fig. 3). Interestingly, more than 40% of the farmers associated the risk of reused wastewater with high chemical contaminants and thus they were not certain on safety of such water for crop irrigation.

The observed awareness and perception under this particular study seemed to be influenced by level of education and experience in production of vegetables farmers had ($P<0.05$).

Although there were differences in awareness and perception on using recovered wastewater for vegetable irrigation between male and female, the difference was not statistically significant ($P>0.05$) (Table 2). This is conversely differ from observation made in a study carried out in Morogoro, Tanzania which reported differences in aversion between male and female of which female found to be less aversive to most occupational health risk than male (Mayilla *et al*., 2017). Also the results differ from that reported from a study conducted in United States of America about perception of health risk associated with consumption of vegetables irrigated with wastewater (Robinson *et al*., 2012). This survey further noted that eating of raw vegetables such as salads and raw carrots was a common practice among vegetable farmers in the study area (Fig. 5). In fact, more than 80% of the farmers participated in the survey revealed consumption of salad and raw carrots to be a common practice (Fig. 5).

Nevertheless, about 88% of the farmers obtained these vegetables they consume from various sources (such as their own farms, buying from local market or from vendors in the streets), whereas 98% of them do not ask the origin of irrigation water for vegetables they buy. It has been reported that contaminated vegetables irrigated by reused wastewater transmits pathogenic agents to human (Hajjami *et al*., 2012; Nwele *et al*., 2013; Sabbahi *et al*., 2018). This is also supported by the reported increase in cases of parasitic diseases experienced by the majority of respondents (83%) in this study (Fig. 4).

It has been noted that smallholder farmers in Mbeya grow mixed type of vegetables and employed spray and sprinkler irrigation methods. The used irrigation methods had been suggested to have highest potential risk of transmission of pathogens to crop surfaces, since water are applied to edible parts of crops and allows a wider movement of pathogens (FAO, 1992; FAO, 2002). Therefore, the results suggest to be one of the reasons for high concentration of intestinal parasitic helminths eggs detected from irrigated vegetables in the surveyed area.

WHO put forward guidelines to safeguard public health for the reuse of wastewater for agriculture purposes (WHO, 2006). In this study, the mean eggs concentration of some parasitic helminths eggs was higher than the WHO recommended guideline values in both effluent of WWTPs and PSCW (Fig. 6 and 7). The results are in agreement with a study conducted in other countries such as Ghana (Amoah *et al*., 2016), Nigeria (Nwele *et al*., 2013; Okojokwu *et al*., 2014) and Morocco (Hajjami *et al*., 2013). The results implies that reused water used by smallholder farmers in the study area does not conform to the standard set by WHO and thus posing risk for transmission of some parasitic helminths such as *A. lumbricoides*, *A. duodenale* and *Taenia sp*. which may cause diseases like cysticercosis to human and livestock (Räisänen *et al.*, 1985; Ensink *et al.*, 2008).

There was variation of mean monthly eggs concentration for all intestinal parasitic helminths eggs identified in irrigation water, with the effluent water from WWTPs showing higher concentrations than PSCW (Fig. 5 and 6). The results observed may be attributed by the effect of dilution since recovered wastewater from Kalobe WWTPs in

Mbeya are channeled to Meta stream which is used for irrigation in vegetable fields downstream. The same result pattern have been observed in experimental study done by Balkhair *et al*. (2014). However, the results showed that there was not statistically significant difference in eggs concentration before and after dilution (x^2=21.2, P>0.05). This implies that even though eggs concentration in recovered wastewater seems to decrease downstream yet the risk of infection remain viable. In fact, parasitic helminths require low dose as few as one ova/egg of infective stage to cause the infection to the new host (Jimenez, 2007). In addition the control water from Ilolo stream which is the upstream of Meta stream before joining with effluent water of Kalobe WWTPs contained none of the parasitic helminths which implies that the parasitic eggs identified originated from WWTPs.

Based on conventional microscopic observation, intestinal parasitic nematodes (*Ascaris sp*., Hookworms, *Strongyloides sp*., *Enterobius sp*. and *Trichuris sp*.) and relatively few cestodes (*Taenia sp*. and *Hymenolepsis sp*.) eggs were recovered from effluent water from WWTPs, PSCW and vegetables samples collected (Fig. 6 and 7, and Table 8). These findings are in agreement with other studies in reused wastewater for irrigation, which noted a higher prevalence of STH in wastewater used for irrigation in Egypt (Stott *et al*., 2003) in Malaysia and Indonesia (Loganathan *et al*., 2016). On the other hand cestodes especially *Taenia spp*. was reported to be highly prevalent in Morocco (Hajjami *et al*., 2013), it comprised 11.1% of the total parasite load in vegetable samples collected. High rate of Taeniid eggs recovery from reused wastewater and vegetable samples collected suggested that Mbeya is highly prevalent area for Taeniasis in human. Hookworm sp. was the second most prevalent parasite identified in water samples from both WWTPs and PSCWs. These results showed that the prevalence of Hookworms species in the current study was higher than that reported among reused wastewater in Iran (Mahvi and Kia, 2006). In particular, among wastewater samples, our results were similar to that reported from India (Gupta *et al*., 2009).

The eggs of intestinal helminths were found in vegetable samples collected from fields irrigated by reused wastewater (Table 8). This study noted that vegetable samples collected in December, 2018 and January, 2019 were directly irrigated with PSCW which indicated the *Ascaris sp*. had highest rate of recovery (4.88 eggs/100g) followed by Hookworm (4.31 eggs/100g) and *Taenia sp*. (4.69 eggs/100g) of which *Trichuris sp*. had the lowest rate of eggs recovery (1.13 eggs/100g). The results are in agreement with other studies (Loganathan *et al*., 2016). However, results showed the drastic drop of trend in parasitic helminths eggs recovery to mean of 0 eggs/100g of vegetable for most of parasites. This may be due to the fact that, starting from late January to June is the rainy season for Mbeya region which imply that farmers were no longer using polluted stream water for vegetable irrigation. Additionally, for loosely adhered parasitic helminths eggs on the vegetable due to irrigation with contaminated water may be flashed away by rains. Thus result into low concentration of parasitic helminths eggs.

In fact, distribution of the recovered helminthes eggs is highly dependent on the frequency of irrigation using recovered wastewater from PSCW which is influenced by environmental factors such as rainfall and humidity. Therefore the current findings may be due to fact that vegetables were not irrigated at all, mostly depended on rain water and humid weather during that season. Contamination with *Taenia sp*. eggs in reused wastewater and irrigated vegetable samples from field areas were prominently noted in this study. As matter of fact this suggest Taeniasis infection status of population of people around Mbeya who drain their wastes in Mbeya city sewerage system. In addition, the contamination of wastewater and irrigated vegetable by *Taenia sp*. eggs were seen to be proportional with prevalence of human *Taeniasis* in endemic areas; thus, Taeniasis/cysticercosis were noted to be among the important parasitic disease of public health in the study area. Eating of raw food such as fresh vegetables as a risk factor which influence prevalence of Taeniasis/cysticercosis in endemic areas was also the reason reported by Carabin *et al*.(2015) and Sánchez *et al*. (1998).

The identification of helminths based on the morphology of the eggs can be satisfied up to the genus level, and the method has been used within parasitology laboratories for diagnosis purposes for many decades. Morphological observations coupled with molecular techniques have been found to be the best methods to identify the parasite species to date (Mathis and Deplazes, 2006). Among 30 Taeniid eggs positive samples by microscopic examination, 50% (n=15) of them were confirmed *T. solium* PCR positive (Fig. 8). Among the two strains of *T. solium*, the American/African origin strain was the only detected in the positive samples. The results reveal the possible channel which influence high prevalence of Taeniasis/cysticercosis in human and pigs in the study area (Komba, 2008).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study the following conclusions are made:

i. Farmers were aware of the health risks of consumption of contaminated crops irrigated with reused wastewater, although, majority of them failed to explain the link between reused wastewater for crop irrigation and associated health risks like enteric disease.

ii. The perception regarding consumption of vegetable irrigated with reused wastewater seems to be influenced by level of education and length of time (years) involved in crop production using recovered wastewater.

iii. Recovered wastewater from Kalobe WWTPs was contaminated with high levels of intestinal helminths eggs (especially *Ascaris sp.*, hookworms, *Strongyloides sp*., *Taenia sp*. and *Hymenolepis sp*.) than levels recommended by WHO. Thus, release of such water in nature such as near streams and rivers is likely to jeopardize the health of people who came into contact either direct or indirect.

iv. Vegetables irrigated using recovered wastewater was contaminated with parasitic intestinal helminths risking the health of consumers.

v. PCR techniques confirmed 50% of taeniid eggs to be *T. solium* proving the potential link between reused wastewater irrigated vegetables and transmission of *T. solium* cysticercosis in the study area.

6.2 Recommendations

According to the findings from this study, the following are recommendations;

i. Training to increase community awareness and understanding on the link of health problems and consumption of contaminated vegetables irrigated with reused wastewater should be provided particularly to farmers and consumers.

ii. More efforts should be devoted to manage further wastewater treatment before channeled for being reused for other human activities.

iii. Frequent parasitological and microbial analysis should be encouraged for monitoring contamination levels to safeguard the public health.

iv. More studies should be encouraged on reused wastewater chemical contaminations and other microbial pathogens profiles using modern techniques such as molecular methods. These will help to provide evidences of infection risks due to contaminated reused wastewater and thus pave the ways of intervention to control those infections.

REFERENCES

Adegoke, A. A., Amoah, I. D., Stenström, T. A., Verbyla, M. E. and Mihelcic, J. R. (2018). Epidemiological Evidence and Health Risks Associated With Agricultural Reuse of Partially Treated and Untreated Wastewater: A Review. *Frontiers in Public Health* 6(337): 1-20.

Amoah, I. D., Abubakari, A., Stenström, T. A., Abaidoo, R. C. and Seidu, R. (2016). Contribution of Wastewater Irrigation to Soil Transmitted Helminths Infection among Vegetable Farmers in Kumasi, Ghana. *PLoS Neglected Tropical Diseases* 10(12): 1–12.

Amoah, P., Drechsel, P., Abaidoo, R. C. and Henseler, M. (2007). Irrigated urban vegetable production in Ghana: Microbiological contamination in farms and markets and associated consumer risk groups. *Journal of Water and Health* 5(3): 455-466.

Bahri, A., Drechsel, P. and Brissaud, F. (2010). Water reuse in Africa: challenges and opportunities. *International Water Management Institute*. pp.1–16.

Balkhair, K. S., El-Nakhlawy, F. S., Al-Solimani, S. G. and Ismail, S. M. (2014). Effect of diluted wastewater and irrigation systems on the yield and contamination of vegetable crops in arid region. *Journal of Food, Agriculture and Environment* 12(2): 579–586.

Blumenthal, U. J. and Peasey, A. (2002). Critical review of epidemiological evidence of the health effects of wastewater and excreta use in agriculture. *In World Health Organization*. [http://www.who.Int/water_sanitationhealth/wastewater/ %0Awho criticalrev.pdf] site visited on 22 June, 2019.

Braae, U.C., Magnussen, P., Lekule, F., Harrison, W. and Johansen, M. V. (2014). Temporal fluctuations in the sero-prevalence of *Taenia solium* cysticercosis in pigs in Mbeya Region, Tanzania. *Parasites and Vectors* 7(574): 1–7.

Cappello, M. (2004). Global health impact of soil-transmitted nematodes. *Pediatric Infectious Disease Journal* 23(7): 663–664.

Carabin, H., Millogo, A., Cissé, A., Gabriël, S., Sahlu, I., Dorny, P. and Ganaba, R. (2015). Prevalence of and Factors Associated with Human Cysticercosis in 60 Villages in Three Provinces of Burkina Faso. *PLoS Neglected Tropical Diseases* 9(11): 1–20.

Coral, M. R., Garcı, H., Devleesschauwer, B., Benı, W., Rodrı, S. and Praet, N. (2014). Incidence of Human *Taenia solium* Larval Infections in an Ecuadorian Endemic Area : Implications for Disease Burden Assessment and Control. *PLOS Neglected Tropical Diseases* 8(5): e2887.

Crompton, D.W.T. and Nesheim, M.C. (2002). Nutritional Impact of Intestinal Helminthiasis during the Human Life Cycle. *Annual Review of Nutrition* 22(1): 35–59.

De Silva, N. R., Brooker, S., Hotez, P. J., Montresor, A., Engels, D. and Savioli, L. (2003). Soil-transmitted helminth infections: Updating the global picture. *Trends in Parasitology* 19(12): 547–551.

Diseases, T., Etiology, T. H. E. and Epilepsy, O. F. (1994). Relationship between Epilepsy and Tropical Diseases. *Raves Press Limited* 35(1): 89–93.

Drechsel, P., Graefe, S., Sonou, M. and Cofie, O. O. (2006). Informal irrigation in urban West Africa: An overview. Colombo, Sri Lanka: International Water Management Institute, Research report 102. 40pp.

Dyachenko, V., Beck, E., Pantchev, N. and Bauer, C. (2008).Cost-effective method of DNA extraction from taeniid eggs. *Parasitology Research* 102: 811–813.

Embrey, M. (2003). Subpopulations susceptible to waterborne diseases are surprisingly diverse. *Journal of American Water Works Association* 95(3): 34–37.

Ensink, J. H. J., Blumenthal, U. J. and Brooker, S. (2008). Wastewater quality and the risk of intestinal nematode infection in sewage farming families in Hyderabad, India. *American Journal of Tropical Medicine and Hygiene* 79(4): 561–567.

Ensink, J. H. J., Van Der Hoek, W., Matsuno, Y., Munir, S. and Aslam, M. R. (2002). Use of Untreated Wastewater in Peri-Urban Agriculture in Pakistan: Risks and Opportunities. *Water Management International Institute* 64(1): 1 – 8.

Feevale, U., Hamburgo, N., Feevale, U., Hamburgo, N., Federal, U., Alegre, P. and Alegre, P. (2012). Assessment of enteric viruses in a sewage treatment plant located in Porto Alegre, southern Brazil. *Brazil Journal of Biology* 72(4): 839–846.

Gaasenbeek, C.P.H. and Borgsteede, F.H.M. (1998). Studies on the survival of *Ascarissuum* eggs under laboratory and simulated field conditions. *Veterinary Parasitology* 75: 227–234.

Gabriël, S., Dorny, P., Mwape, K. E., Trevisan, C., Braae, U. C., Magnussen, P. and Johansen, M. V. (2017). Control of *Taenia solium* taeniasis / cysticercosis: The best way forward for sub-Saharan Africa? *ActaTropica* 165: 252–260.

Gordon, C.A., Gray, D.J., Gobert, G.N. and McManus, D.P. (2011). DNA amplification approaches for the diagnosis of key parasitic helminth infections of humans. *Molecular and Cellular Probes* 25(4): 143–152.

Goussanou, J. S. E., Kpodekon, M. T., Youssao, A. K. I., Farougou, S. and Korsak, N. (2014). Epidemiological tools for effective surveillance of porcine cysticercosis in Africa. *Veterinary World* 7(3): 125–134.

Gupta, N., Khan, D. K. and Santra, S. C. (2009). Prevalence of intestinal helminth eggs on vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. *Food Control* 20(10): 942–945.

Hajjami, K., Ennaji, M. M., Fouad, S., Oubrim, N. and Cohen, N. (2013). Wastewater Reuse for Irrigation in Morocco: Helminth Eggs Contamination′s Level of Irrigated Crops and Sanitary Risk (A Case Study of Settat and Soualem Regions). *Journal of Bacteriology and Parasitology* 4(1): 1–5.

Jaramillo, M. F. and Restrepo, I. (2017). Wastewater reuse in agriculture: A review about its limitations and benefits. *Sustainability (Switzerland)* 9(10): 17–34.

Jemli, M., Sabbahi, S. and Ayed, L. Ben. (2015). Performance of Urban Wastewater Treatment of water. *International Journal of Water and Wastewater Treatment* 1(1): 25 – 40.

Jha, S., Rollins, M. G., Fuchs, G., Procter, D. J., Hall, E. A., Cozzolino, K. and Walsh, D. (2017). Clinical symptoms, diagnosis and treatment of neurocysticercosis. *Lancet Neurology* 546(7660): 651–655.

Jiménez, B. (2006). Irrigation in Developing Countries Using Wastewater. *International Review for Environmental Strategies* 6(2): 229–250.

Jimenez, B. (2007). Helminth ova removal from wastewater for agriculture and aquaculture reuse. *Water Science and Technology* 55: 485–493.

Jimenez, B. and Asano, T. (2012). Water Reuse: An International Survey of current practice, issues and needs. *International Water Management* publishing: London, United Kingdoms. pp. 1-592.

Jiménez, B., Maya, C., Barrios, J. A. and Navarro, I. (2017). Helminths and their Role in Environmental Engineering. *Intech Open*. [https://org/10.13140/RG. 2.1.2934 .5365]. Site visited on 3/2/2018.

Karamon, J. and Cencek, T. (2018). Identification and control of sources of *Taeniasolium* infection- the attempts to eradicate the parasite. *Journal of Veterinary Resources* 62(11): 27–34.

Karan, A., Chapman, G. B. and Galvani, A. (2012). The influence of poverty and culture on the transmission of parasitic infections in rural Nicaraguan villages. *Journal of Parasitology Research* 2012: 1-12.

Kayombo, S., Mbwette, T. S. A., Katima, J. H.Y., Ladegaard, N. and Jørgensen, S. E. (2005). Waste stabilization ponds and constructed wetlands design manual. *UNEP-IETC with the Danish International Development Agency* (*Danida*) pp. 1–59.

Keraita, B., Abaidoo, R., Beernaerts, I., Koo-Oshima, S., Amoah, P., Drechsel, P. and Konradsen, F. (2012). Safe Re-use Practices in Wastewater-Irrigated Urban Vegetable Farming in Ghana. *Journal of Agriculture, Food Systems, and Community Development* 2(4): 147–158.

Kimbi, E. C. and Lekule, F. P. (2016). Smallholder Pig Marketing Systems in the Southern Highlands of Tanzania. *Journal of Natural Sciences Research* 6(14): 87–98.

Konaté, Y., Maiga, A. H., Basset, D., Picot, B. and Casellas, C. (2013). Occurrence, removal and accumulation in sludge of protozoan cysts and helminth eggs in a full-scale anaerobic pond in Burkina Faso. *Water Science and Technology* 67(1): 193–200.

Liu, C., Luo, R., Yi, H., Zhang, L., Li, S. and Bai, Y. (2015). Soil-Transmitted Helminths in Southwestern China: A Cross-Sectional Study of Links to Cognitive ability, Nutrition, and School Performance among Children. *PLoS Neglected Tropical Diseases* 9(6): 1–16.

Loganathan, R., Agoes, R. and Arya, I. F. D. (2016). Vegetables contamination by Parasitic Helminth Eggs in Malaysia and Indonesia. *Althea Medical Journal* 3(2): 190–194.

Mahvi, A. H. and Kia, E. B. (2006). Helminth eggs in raw and treated wastewater in the Islamic Republic of Iran. *Eastern Mediterranean Health Journal* 12(2): 137–143.

Mathis, A. and Deplazes, P. (2006). Copro-DNA tests for diagnosis of animal taeniid cestodes. *Parasitology International* 55(2): 56-78.

Mayilla, W., Keraita, B., Ngowi, H., Konradsen, F. and Magayane, F. (2017). Perceptions of using low-quality irrigation water in vegetable production in Morogoro, Tanzania. *Environment, Development and Sustainability* 19(1): 165–183.

Mkupasi, E. M., Ngowi, H. A. and Nonga, H. E. (2011). Prevalence of extra-intestinal porcine helminth infections and assessment of sanitary conditions of pig slaughter slabs in Dar es Salaam city. *Tanzania. Tropical Animal Health and Production* 43(2): 417–423.

Mojid, M. A., Wyseure, G.C.L., Biswas, S.K. and Hossain, A. B. M. Z. (2010). Farmers' perceptions and knowledge in using wastewater for irrigation at twelve peri-urban areas and two sugar mill areas in Bangladesh. *Agricultural Water Management* 98(1): 79–86.

Mwanjali, G., Kihamia, C., Kakoko, D. V. C., Lekule, F., Ngowi, H., Johansen, M. V. and Willingham, A. L. (2013). Prevalence and Risk Factors Associated with Human *Taenia Solium* Infections in Mbozi District, Mbeya Region, Tanzania. *PLoS Neglected Tropical Diseases* 7(3): e2102.

Ngowi, H. A., Carabin, H. and Kassuku, A. A. (2008). A health-education intervention trial to reduce porcine cysticercosis in Mbulu District, Tanzania. *Preventive Veterinary Medicine* 85: 52–67.

Nwele, D. E., Uhuo, A. C., Okonkwo, E. C., Ibiam, G. A., Onwe, C. S., Ugwu, J. I. and Onwe, J.C. (2013). Parasitological examination of Ava stream used in irrigation in Enugu State, South-Eastern Nigeria : An implication for helminth transmission. *Journal of Parasitology and Vector Biology* 5(8):112–115.

Ofred, J. M., Robinson, H. M., Lughano, J. M. K., Anita, F. and Anders, D. (2016). Removal of Escherichia coli in treated wastewater used for food production in Morogoro, Tanzania. *African Journal of Microbiology Research* 10(33): 1344–1350.

Okoh, A. I., Sibanda, T. and Gusha, S. S. (2010). Inadequately treated wastewater as a source of human enteric viruses in the environment. *International Journal of Environmental Research and Public Health* 7(6): 2620–2637.

Okojokwu, O., Inabo, H. and Yakubu, S. (2014). Parasitological Profile of Raw Wastewater and the Efficacy of Biosand Filter in Reduction of Parasite Ova and Cysts. *Journal of Applied Sciences and Environmental Management* 18(1): 5-9.

Outwater, A. B., Outwater, A. H. and Pemba, S. (2013). Dissemination of the sustainable wastewater technology of constructed wetlands in Tanzania: Water - related Diseases of People using Municipal Wastewater: Risks, Exposure, Effects on Health and Control Approaches in Tanzania. [https://org/10.13140/RG. 2.1.2934.5365]. Site visited on 22/6/2019.

Pescod, M. B. (1992). Wastewater treatment and use in agriculture - FAO irrigation and drainage. In Food and Agriculture Organization of the United Nations. Rome 47pp. [https://org/M-56]. Site visited on 2/6/2019.

Räisänen, S., Ruuskanen, L. and Nyman, S. (1985). Epidemic ascariasis-evidence of transmission by imported vegetables. *Scandinavian Journal of Primary Health Care* 3(3): 189–191.

Robinson, K.G., Robinson, C. H., Raup, L. A. and Markum, T. R. (2012). Public attitudes and risk perception toward land application of biosolids within the south-eastern United States. *Journal of Environmental Management* 98(1): 29–36.

Sabbahi, S., Trad, M., Ben Ayed, L. and Marzougui, N. (2018). Occurrence of intestinal parasites in sewage samples and efficiency of wastewater treatment systems in Tunisia. *Water Quality Research Journal* 53(2): 86–101.

Samson, S., Mdegela, R. H., Permin, A., Mlangwa, J. E. D. and Mahonge, C. P. (2017). Obstacles to low quality water irrigation of food crops in Morogoro, Tanzania. *Journal of Sustainable Development* 10(2): 1–12.

Sánchez, A. L., Medina, M. T. and Ljungström, I. (1998). Prevalence of taeniasis and cysticercosis in a population of urban residence in Honduras. *Acta Tropica* 69(2): 141–149.

Savioli, L. and Albonico, M. (2004). Disease watch: Focus: Soil-transmitted helminthiasis. *Nature Reviews Microbiology* 2(8): 618–619.

Siza, J. E., Kaatano, G. M., Chai, J. Y., Eom, K. S., Rim, H. J., Yong, T. S. and Changalucha, J. M. (2015). Prevalence of schistosomes and soil-transmitted helminths and morbidity associated with schistosomiasis among adult population in Lake Victoria basin, Tanzania. *Korean Journal of Parasitology* 53(5): 525–533.

Stott, R., May, E. and Mara, D. D. (2003). Parasite removal by natural wastewater treatment systems: Performance of waste stabilisation ponds and constructed wetlands. *Water Science and Technology* 48(2): 97–104.

Tanji, K. K. and Kielen, N. C. (2002). Agricultural drainage water management in arid and semi-arid areas. FAO Irrigation and drainage paper 61. In FAO, Rome. [http://www.fao.org/3/a-ap103e.pdf]. Site visited on 14/4/2019.

Tilley, E., Lüthi, C., Morel, A., Zurbrügg, C. and Schertenleib, R. (2014). Compendium of Sanitation Systems and Technologies. 2nd Revised Edition.*In Swiss Federal Institute of Aquatic Science and Technology* (Eawag) (2nd Revise). [https://doi.org/SAN-12]. Site visited on 20/3/2019.

Torgerson, P. R., Devleesschauwer, B., Praet, N., Speybroeck, N., Willingham, A. L., Kasuga, F. and de Silva, N. (2015). World Health Organization Estimates of the Global and Regional Disease Burden of 11 Foodborne Parasitic Diseases, 2010: A Data Synthesis. *PLoS Tropical Medicine* 12(12): 1–22.

URT (2013). 2012 Population and Housing Census Population Distribution by Administrative areas. *National Bureau of Standard*. Dar-es-Salaam, Tanzania 45pp.

URT (2007). National Sample Census of Agriculture 2002/2003: Regional report, Mbeya region. Retrieved from [http://www.fao. org/tempref/AG/Reserved/PPLPF/

ftpOUT/GLiPHA/DATA/Queue/Working/tanzania/MBEYAREGION REPORT .pdf]. Site visited on 7/6/2019.

Weckenbrock, P., Evans, A., Majeed, M. Q., Ahmad, W., Bashir, N. and Drescher, A. (2011). Fighting for the right to use wastewater: What drives the use of untreated wastewater in a peri-urban Village of Faisalabad, Pakistan? *Water International* 36(4): 522–534.

Wilson, R. T. and Swai, E. S. (2014). Pig Production in Tanzania : a Critical Review. *Tropicultura* 32(1): 46–53.

WHO (1996). Analysis of Wastewater for Use in Agriculture – A Laboratory Manual of Parasitological and Bacteriological Techniques. Ayres, G. R. M. and Mara, D.D. University of Leeds, England. 34pp.

WHO (2006). Safe Use Water Guidelines for the Safe Use of Wastewater, Excreta and Grey. *World Health Organization* 1: 1-204.

Zacharia, A., Outwater, A. H., Ngasala, B. and Deun, R. Van. (2018). Pathogenic Parasites in Raw and Treated Wastewater in Africa: A Review. *Resources and Environment* 8(5): 232–240.

APPENDIXES

Appendix 1: Sample questionnaire

1. Date …………………………………………
2. Questionnaire number ……………………
3. Location (District/city, Ward, Village/street)

   ……………………………………………

Part A: Personal particulars

Respondent Identification …………………………………………………

<table>
<tr><td rowspan="2">Sex</td><td colspan="4">Male</td><td></td></tr>
<tr><td colspan="4">Female</td><td></td></tr>
<tr><td rowspan="4">Age</td><td colspan="4">Below 18 years</td><td></td></tr>
<tr><td colspan="4">Between 18-40 years</td><td></td></tr>
<tr><td colspan="4">Between 41-60 years</td><td></td></tr>
<tr><td colspan="4">Above 60 years</td><td></td></tr>
<tr><td rowspan="2">Marital status</td><td colspan="4">Married</td><td></td></tr>
<tr><td colspan="4">Single</td><td></td></tr>
<tr><td></td><td colspan="4">Separated</td><td></td></tr>
<tr><td></td><td colspan="4">Widow</td><td></td></tr>
<tr><td rowspan="4">Educational level</td><td colspan="4">No formal education</td><td></td></tr>
<tr><td colspan="4">Primary education</td><td></td></tr>
<tr><td colspan="4">Secondary education</td><td></td></tr>
<tr><td colspan="4">Higher education</td><td></td></tr>
<tr><td rowspan="2">Important Income generating activities</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
</table>

1. Crop production 2. Livestock production 3. Formal employment 4. Causal labour 5. Others

Household size ………………

Part B: General inquire

I. General information

1. What is your main income generating activity?

   ………………………………………………

2. For how long have you been involving in crop production?

   i. Less than one year

   ii. Between 1-5 years

   iii. More than 5 years

3. What type of crops do you grow and for what purpose?

S/N	Type of crop	Purpose		
		1= For home consumption	2= For sell	3= For home consumption and for sell
1				
2				
3				
4				
5				

II. Vegetable farm and farming Practices

4. Do you think what are the **advantage** and **disadvantage** of using this kind of water for irrigation compared to other type?...

5. Do you require permission to use the water for irrigation? (yes/no)

6. If yes (in question 9. Above), where from……………………………………………

7. What do you do with leftovers after harvesting?
   i. Destroy/through away
   ii. Feed livestock
   iii. Others (specify………….)

8. What type of water do you use for the post-harvest vegetables processing?
   i. Tap water
   ii. The same wastewater
   iii. Ground/borehole water
   iv. River water
   v. Others (specify………….)
9. How do you perceive/regard the use of this sewage water for vegetable irrigation? Explain

   ……………………………………………………………………………………………
10. Is there any health problem for the use of sewage water for vegetable irrigation (Yes/no)
11. If yes, mention some of them…………………………………………………………….

III. Vegetable Consumption practices

12. How frequent do you use vegetables in your household daily meal? ………………
13. Where do you normally get vegetable from?
    i. Your farm
    ii. Buy from local market
    iii. Buy from vendors

iv. Others (specify……………)

14. If you buy from local market or vendors, do you know/ask the water source used to irrigate the vegetables? (Yes/No)
15. If yes (in 13. above), could you continue buying if the water source is wastewater? (Yes/No). Why?..
16. What do you normally do at home to clean these vegetables?
    i. Wash with clean water
    ii. Wash in salt water
    iii. Add a disinfectant
    iv. Others (specify…………………)
17. How often do you consume raw vegetables (consume as salad)?.............................
18. Are you aware of any health risks associated with the consumption of vegetables that are irrigated with wastewater? (yes/no)
19. If yes, what health risks do you know? …………………………………
20. Do you perceive or experienced any negative health problems related to the consumption of vegetables? (Yes/No)
21. If yes (in 19. Above), please mention ……………………………………………
22. Have you had diarrheal after consuming salads? (Yes/No/ cannot remember)
23. How often do you experience diarrheal cases to member(s) of your household?
24. Do you associate diarrhea cases with consumption of vegetables irrigated with wastewater? (Yes/No)
25. If yes explain how?...

Before we windup our discussion, I would like to thank you very much for your cooperation and also to emphasize our agreement that, this discussion is confidential.

Appendix 2: Checklist to guide observational survey in vegetable irrigation practices

Checklist for vegetable irrigation practices

1. Type of water used for irrigation (reused wastewater/raw sewage water)
2. Kind of water used for irrigation (raw wastewater/ treated wastewater from WWTPs/ Polluted streams water/Polluted streams channeled with treated wastewater)
3. For irrigated water, what is the movement speed (High/slow/settled pond)
4. Irrigation practice (Gravity/pumping/open canal flow/by hand/ other forms……..)
5. Type of vegetables mostly cultivated (folded vegetables/unfolded vegetables)