BACTERIAL CONTAMINANTS OF AFRICAN INDIGENOUS LEAFY VEGETABLES AND THEIR ANTIBIOTICS SENSITIVITY CHARACTERISTICS: A CASE STUDY OF MOROGORO MUNICIPALITY, TANZANIA

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

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ABSTRACT

A study was conducted in Morogoro Municipality, Tanzania to investigate the bacterial load found on African indigenous leafy vegetables, their sources and their sensitivity to commonly used antibiotics. A total of 126 samples of fresh African indigenous leafy vegetables (amaranth, nightshade and sweet potato leaves), water used for irrigation from rivers and shallow wells and manure fertilised soils were collected at farm sites and market outlets for bacteriological analysis. Bacterial counts were calculated as colony forming units (CFU) per millilitre (CFU/ml) of vegetable washing. The isolates sensitivity or resistances to antibiotics were determined on Muller – Hinton agar plates by the disk diffusion technique. Data was subjected to Analysis of Variance (ANOVA) using SAS Statistical software. Results showed that microorganisms were abundant on the surfaces of the African indigenous leafy vegetables, with nightshade having 1.8×10^5 CFU/ml while amaranth and sweet potato leaves having 1.7×10^5 CFU/ml and 1.5×10^5 CFU/ml respectively. The water used for irrigation from the lower section of the river had significantly ($P = 0.03$) higher bacterial loads 1.8×10^5 CFU/ml than water from the higher sections. Biochemical tests indicated bacterial isolates from the studied vegetables to be *Escherichia coli, Enterobacter aerogenes, Proteus* spp*, Staphylococcus albus and Bacillus* spp. The results show that *Escherichia coli, Enterobacter aerogenes and Proteus* spp (coliforms) were highly sensitive (>75 %) to enrofloxacin, sulphamethoxazole and ofloxacin antibiotics but resistant to rifampicin. The observed bacterial loads, and sensitivity patterns to commonly used antibiotics reveal the potential adverse health impact of the vegetables on consumers. Proper handling and preparation of vegetables before consumption is highly recommended. Further research covering different African indigenous leafy vegetables in wider agro-ecological areas and sensitivity patterns to commonly used antibiotics is also strongly recommended.

DECLARATION

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

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
AST	Antimicrobial Susceptibility Testing
BA	Blood agar
CFU	Colony Forming Unit
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization
FC	Feacal Coliforms
H_2O_2	Hydrogen Peroxide
ICMSF	International Commission on Microbiological specifications for Food
IMVC	Indole, Methyl red, Voges proskeur and Citrate chemical test
KOH	Potassium Hydroxide
LSD	Least Significant Difference
MC	MacConkey agar
MR	Methyl Red
NEMLIT	National Essential Medicines List
PCR	Polymerase Chain Reaction
SAS	Statistical Analysis Software
STG	Standard Treatment Guidelines
SUA	Sokoine University of Agriculture
USA	United States of America
VRB	Violet Red Bile
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

African indigenous vegetables are important food components for humans in both urban and rural settings (Weinberger and Msuya, 2004). They are a primary source of mineral nutrients, secondary plant metabolites and vitamins that protect the body including prevention of diseases (Sinha *et al*., 2011). The most commonly reported African indigenous vegetables in Tanzania and Morogoro in particular, include: amaranths (in swahili *Mchicha*), nightshade (in Swahili *Mnavu*), pumpkin leaves, radish (in Swahili *Figiri*), jute mallow (in Swahili *Mlenda*), African eggplant (in Swahili *Ngogwe*) and cowpea (Nicodemas, 2013). It is expected that in the coming years, production of these crops will continue to increase because they fit into year-round production systems, their high nutritional value, and their low requirements for irrigation as well as its short maturity period (Prohens and Nuez, 2008).

Green leafy vegetables are easily contaminated with water, animal manure and dust particles rich in microorganisms, including potential human pathogens (Neluheni *et al*., 2007). Berger *et al*. (2010) reviewed disease outbreaks associated with the consumption of green leafy vegetables such as fluted pumpkin, bitter leaves, lettuce, cabbage and cucumber, and reported the most common human pathogens contaminants as *Sallmonella* spp, *Shigella* spp and *Escherichia coli.* In Saudi Arabia, a study was conducted to describe the bacterial load and the occurrence of some disease-causing bacteria on raw vegetables (Watermelon, Spinach, Tomatoes, Lettuce, Cucumber, Cabbage, and Parsley) sold in Saudi markets (Hassan *et al*., 2011). In this study *S. aureus* and *Shigella* spp were the most common bacteria detected. The study reported further that these isolates were resistant to

ampicillin, cephalothin, trimethoprime-sulfamethoxazole, aminoglycosides, tetracycline, fluoroquinolones, amoxycillin-clavulanic acid and chloramphenicol.

Outbreaks of illness associated with consumption of leafy vegetables contaminated with microorganisms and their resistance to antibiotics have been well documented worldwide (Nipa *et al.*, 2011). There is, however, paucity of information on bacterial organisms on African indigenous leafy vegetables and their resistance to antibiotics in Tanzanian cities, like Morogoro, where farming and marketing of amaranth, nightshade and sweet potato leaves is common.

1.2 Problem Statement

Leafy vegetables are an exceptional dietary source of nutrients, micronutrients, vitamins and fibre for human beings, hence vital for health and fitness. These leaves, which promote good health, harbour a wide range of microbial contaminants, thus undermining their nutritional and health benefits, and instead increase consequently outbreaks of human infections associated with their consumption (James, 2006). Studies elsewhere have focused on bacterial load of leafy vegetables such as cabbage, cucumber, carrot and lettuce, where bacterial organisms including *Sallmonella* spp., *Shigella* spp., *E. coli, S. aureus, Streptococcus faecalis and Bacillus* spp., were reported (Brandl and Amundson, 2008; Hassan *et al.*, 2011). The study conducted by Abdullah and Abdulkareem (2010) in Nigeria, emphasised on bacterial load of some fresh leafy vegetables namely, lettuce, cabbage and cucumber, which were collected from different retailers in Sabon Gari market. The result from this study indicated *Bacillus* spp., and *S. aureus* were the predominant bacteria isolated. A study conducted on the occurrence of contamination and antibiotic resistance in *E. coli* isolates from fresh leafy vegetables in Jordanian retail markets demonstrated a widespread of antimicrobial resistance in *E. coli* contaminating

fresh green produce (Burjaq and Shehabi, 2013). The results show further that such contamination may increase the reservoir of antimicrobial resistance in the intestinal tract. Tsado *et al.* (2013) examined the bacterial load of selected leafy vegetables namely fluted pumpkins (*Teliferia occidentalis*), bitter leaf (*Vernonia amygdalina*) and amaranth (*Amaranthus caudatus*) from urban and rural farms and markets in Minna metropolis Niger State, Nigeria. The results indicated that, the bacteria isolated (*Str. faecalis, Str. epidimidis, S. aureus, Bacillus subtilis* and *E. coli*) could pose a serious health hazard to humans if they are consumed raw.

Morogoro urban is notable for its frequent cases of typhoid and other diarrhoeal diseases of water and food origin (Jiwa *et al*., 1991). The perceived sources of such pathogens include: water used for irrigation, fertilizers and the vegetables handling. For example, results of the analysis of the water samples from Mzinga River Catchments of the Southern Dar es Salaam City, Tanzania for total coliform are reported to range from 14.17±4.06 CFU/100ml to 486.80±102.32 CFU/100ml, while faecal coliform ranges from 2.78±1.03 CFU/100ml to 120.36±4.50 CFU/100ml (Saria, 2015). The bacteriological contamination was due to the fact that Mzinga River is passing through a populated urban area thereby more exposed to direct sewage disposal and incoming industrial effluents with probability of people around these areas being prone to water-borne infections such as diarrhea or cholera. However, little information is available on types and exact sources of the major food contaminants, found on indigenous green leafy vegetables, particularly those consumed as salads or prepared snacks (Shackleton *et al.,* 2009). Considering the potential outbreaks of human infections associated with the consumption of leafy vegetables, studies on bacterial load of such vegetables and their associated resistance to antibiotics is of paramount importance. This study has elucidated on the bacterial load of

African indigenous leafy vegetables in selected sources in Morogoro and has established their sensitivity/resistance to commonly used antibiotics.

1.3 Justification

Leafy vegetables are an important dietary source of nutrients, micronutrients, vitamins and fibre for human beings and hence are vital for health and fitness. These leaves promote good health, however, farming management practices make them susceptible to microbial contaminants, which may render them a health risk. Human infections associated with the consumption of leafy vegetables are increasingly being reported (James, 2006). Therefore, evaluation of bacterial load and antimicrobial resistance of the bacterial species found on leafy vegetables is important to support or advice towards health and safe nutrition in Tanzania. In this study the bacterial load of African indigenous leafy vegetables (amaranth, nightshade and sweet potato leaves) in urban farms and market settings were investigated. The study also examined how leafy vegetables serve as vehicles of pathogens associated with food contaminants and their antibiotics resistant in the Morogoro Municipality, as a way to broaden awareness in this region and in other places where the consumption of green vegetables is common. Knowledge on bacterial load of leafy vegetables in Morogoro was envisaged also to contribute towards policy formulation on food safety and to advice on the selection of appropriate antibiotics in situations of disease following consumption of food contaminated with bacterial pathogens.

1.4 Objectives

1.4.1 Overall objective

The overall objective of the study was to establish the bacterial contamination of African indigenous leafy vegetables, their origin and their susceptibility to commonly used antibiotics as an important step towards improving food hygiene.

1.4.2 Specific objectives

i) To determine bacterial load of amaranths, nightshade and sweet potato which are cultivated in different farm sites and sold in the Morogoro Municipality.

ii) To establish the potential sources of the microorganisms found on the leafy vegetables.

iii) To isolate and characterize bacterial species found on African indigenous leafy vegetables at farm sites, market outlets and other sources in Morogoro Municipality.

iv) To carry out antibiotic susceptibility tests of potentially pathogenic bacterial isolates from the leafy vegetables.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Basic Concepts on the Bacterial Load of African Indigenous Leafy Vegetables

2.1.1 African indigenous leafy vegetables

Indigenous vegetables are highly nutritious and hence preferred by their consumers over their exotic counterparts (Rensburg *et al.,* 2004). Exotic vegetables are crops that have been imported and cultivated in a certain region, outside their area of endemicity (Mibei *et al*., 2011). African indigenous vegetables are, however, commonly referred to as crop species genuinely endemic to the African region, or species introduced into a region in Africa where over a period of time have evolved, successfully (Engle and Altoveros, 2000). According to Smith and Eyzaguire (2007), indigenous African vegetables are those vegetable crops that have their natural habitat on sub-Saharan Africa, while the indigenous leafy vegetables were introduced over a century ago and due to long use, have become part of the food culture in the sub-continent.

According to FAO (1988), indigenous leafy vegetables are all categories of plants whose leaves are acceptable and used as vegetables by urban and rural communities through custom, habit and tradition. Indigenous Leafy vegetables are dominated by plant families such as those of the amaranth (*Amaranthaceae*), nightshade (*Solanaceae*), sunflower (*Asteraceae*), pumpkin (*Cucurbitaceae*), cabbage (*Brassicaceae*), sweet potato leaves and jute (*Tiliaceae*). Different communities have different practises, which vary in harvesting and cooking techniques to either reduce negative characteristics or to enhance the more desirable taste traits (Imbumi and Maundu, 2008). Indigenous leafy vegetables are also defined as wild plants, or semi-domesticated species that are part of traditional diets and

may often be relied upon as foods during periods of crop failure or famine (Guarino, 1997). Gockowski *et al.* (2003) define indigenous leafy African vegetables as those leafy green vegetables that have been originally domesticated or cultivated in Africa for the last several centuries. Rensburg *et al.* (2007) define indigenous leafy vegetables as those plants whose origin is on the continent, or those which have such a long history of cultivation and domestication to African conditions and use that they have become indigenized. Studies in Tanzania show that most of the indigenous vegetables are preferred by 50 to 90% of the people living in urban areas like Morogoro and Dar es Salaam; with amaranth being the most commonly consumed and fully domesticated (Lyimo *et al.*, 2003).

Indigenous leafy vegetables provide a low-cost quality nutrition for large parts of the population in both rural and urban areas (Chweya and Eyzaguirre, 1999). In fact, almost all of these vegetables are good sources of micronutrients including: iron and calcium as well as vitamins A, B complex, C and E. One example is the amaranth, which contains more of these nutrients compared to a typical exotic leafy vegetable such as white cabbage (Weinberger and Msuya, 2004). The study by Backeberg (2013) showed that the indigenous leafy vegetables are more drought and heat tolerant than exotic vegetables, whereas amaranth being the most heat tolerant crop. Gotor and Irungu (2010), collected sufficient data which confirmed that regular consumption of African leafy vegetables can assist in balancing diets by adding essential macronutrients, particularly betacarotene and iron. Several studies done in Kenya have shown that African indigenous leafy vegetables have increasingly become commercially important in Kenya over the last 15 years, where they have increasingly featured in both formal and informal markets in Nairobi and its neighbouring areas (Mwaura *et al.*, 2014). In this study, African indigenous leafy vegetables including amaranths, nightshade and sweet potato leaves were analysed for

surface bacterial contamination as an important step towards improving food hygiene particularly in urban areas.

2.1.2 Bacterial load or bacterial count

Bacterial load, also known as bacterial count, is a procedure that detects all viable microorganisms that could grow aerobically or anaerobically on plate count agar at appropriate incubation condition, usually 37ºC in 48 hours (Environmental Laboratory, 2011). It is a measurable quantity of bacteria in an object, organism, or organism compartment. The bacterial load tests could reflect the general hygiene condition of a sample (Reynolds, 2011).

Microbiological control is important in food industry to prevent food borne diseases. Therefore, the present investigation was undertaken to assess the microbiological quality of African indigenous leafy vegetables collected from several sites in Morogoro Municipality by examining their microbial load.

2.2 Bacterial Species Commonly Found On Leafy Vegetables

Leafy vegetables produced in a natural environment are vulnerable to contamination by human pathogens including *Salmonella* spp, *Shigella* spp, *Escherichia coli* 0157:H7, and *Staphylococcus aureus* (Elhariry, 2011). A study conducted in India revealed the presence of *Escherichia coli, Enterobacter aerogenes, Pseudomonas* spp., *Staphylococcus aureus, Salmonella* spp. and *Shigella* spp. on vegetable salads (Tambekar and Mundhada, 2006). Similar findings were reported by Tsado *et al*. (2015) in Nigeria. Contaminated green vegetables are often a source of bacterial and parasitic outbreaks in humans, involving *E. coli* and *Salmonella*. Dada and Olusola Makinde (2015) conducted a laboratory investigation on six different leafy vegetable samples namely *Amaranthus cruentus*

(amaranth), *Talinum triangulare* (waterleaf), *Solanecio biafrae* (worowo), *Brassica olerecea* (cabbage) and *Lactuca sativa* (lettuce) which were purchased from retailers in the main market, Akure, Nigeria and succeeded to isolate and identify seven bacteria belonging to different species including: *Staphylococcus aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Serratia marcescens, Salmonella typhi* and *Proteus vulgaris.* In this study, it was reported that the total bacterial count ranged from 3.2×10^6 to 7.2×10^6 cfu/g for samples washed with distilled water and 1.6×10^6 to 4.8×10^6 cfu/g for samples washed with physiological saline. Microorganisms isolated from *Amaranthus cruentus* (amaranth) were *Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, Escherichia coli, Rhizopus stolonifera and Saccharomyces* spp.

Epidemiological evidence suggests that leafy vegetables are the second highest risk factor for bacterial infections and the most frequently reported bacteria include: *Campylobacter* (Evans *et al.*, 2003), *Listeria monocytogenes* (Farber *et al.*, 1998), *Escherichia coli* 0157: H7 (Stopforth *et al.*, 2004), *Salmonella* (Doran *et al.*, 2004), *Vibrio cholera* (Shaval *et al.*, 1989) and *Bacillus* spp. (Portnoy *et al.*, 1976). A study conducted in Melka Hida and Wonji Gefersa farms, Ethiopia reported high frequency of contamination of vegetables (spinach, lettuce, cabbage) samples from both farms with *Escherichia coli, Kelbsiella* spp, *Enterobacter* spp, and *Citrobacter* spp (Benti *et al.,* 2014). The study showed a higher aerobic mesophilic bacterial count of 2.2×10^8 and 2.0×10^8 CFU/g, for spinach sampled from Melka Hida and Wonji Gefersa vegetable farms, respectively. The highest total coliform count (6.6×10^6) was also recorded from lettuce in Melka Hida vegetable farm. The mean faecal coliform values of vegetable types exceeded levels of the International Commission on Microbiological Specifications for Foods (ICMSF) recommended by the United Nation (ICMSF, 2006).

The high microbial contamination rates associated with these vegetable samples insinuated from poor water quality for irrigation employed in the overall production of vegetables in the study area. In Kano Metropolis, Nigeria, Taura and Habibu (2009), reported a high frequency of bacterial species contamination of leafy vegetables, namely lettuce, cabbage and spinach. The most common bacterial species isolated were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus* spp and *Streptococcus*, spp. The results of this study demonstrated that out of the samples examined, *Staphylococcus* spp accounted for a high percentage of occurrence with, (42.5 %) followed by *Escherichia coli* (27.9 %), *Pseudomonas aeruginosa* (22 %) and *Streptococcus* spp (7.5 %). In all the samples examined, cabbage was found to be the most contaminated, followed by the lettuce and the spinach. The study concluded that consumption of these types of vegetables, if not prepared hygienically could pave way for ingestion of considerable numbers of human pathogenic bacteria which will ultimately result in the establishment and manifestation of diseases. From the reviewed literature, there is paucity of information on bacterial organisms associated with African indigenous leafy vegetables, whose sources could be related to the water used for irrigation or the manure applied for fertilization. Their sensitivity/resistance to antibiotics is of particular interest in Tanzania where farming and marketing of African indigenous a leafy vegetable is a common practice, particularly in urban and peri urban areas.

2.3 Major Sources of Contamination of Leafy Vegetables

Over the last decades, demand on food safety has increased, thus stimulating research with respect to consumption of food contaminated with pathogenic microorganisms (Benti *et al*., 2014). Vegetables are produced in significant quantities both in urban and peri urban areas. Several studies have revealed the threats caused by vegetables contaminated with pathogens (FAO/WHO, 2008; Denis *et al*., 2016). A wide range of leafy greens, including

lettuce and spinach, are well recognised as potential sources of bacterial infections (FAO/WHO, 2008). The USA has experienced several outbreaks attributed to leafy vegetables, including the 2006 outbreak of *E. coli* O157:H7 infection, linked to the consumption of spinach which resulted in almost 200 cases of food poisoning and three deaths (Wendel *et al.*, 2009). Watermelons from Brazil were implicated in a multi-country outbreak of *Salmonella* infection in Europe, with 63 confirmed cases of food poisoning (Machado *et al.*, 2006). The most common sources of microorganisms that contaminate leafy vegetables include air, soil, farm pests (nematodes), handlers and irrigation water (AVRDC, 1986). Leafy vegetables can become contaminated while growing in the field or during harvest, handling, processing, distribution and use (Beuchat, 1998). Any microbial contamination in leafy vegetables is commonly associated with the environment through which the product has passed (Taura and Habibu, 2009).

Several studies have listed the potential sources of vegetable contamination to be in the production chain, including pre harvest (in the field) and post-harvest phases (Tables 1 and 2). Water is the most important source of contamination of leafy vegetables in the field (Berger *et al.*, 2010). For example, a study conducted in the Loskop dam irrigation scheme in the Mpumaplanga Province, South Africa, examined the presence of total coliforms, faecal coliforms, *E. coli, L. monocytogenes, Salmonella* spp., *Enterococcus* spp, *S. aureus*, aerobic spore formers, anaerobic spore formers and aerobic colony counts (Ijabadeniyi *et al.*, 2012) in water from two rivers that feed the Loskop dam and the water from the Loskop canal system which is used to irrigate broccoli and cauliflower vegetables. In that study, it was reported that levels of faecal coliforms and *E. coli* were higher than the WHO standard of ≤1000 faecal coliforms (FC) per 100ml). *Staphylococcus aureus,* intestinal enterococci, *Salmonella* spp and *L. monocytogenes* were recovered from the two rivers and from the canal. The bacterial pathogens isolated from the three water sources were

also isolated from the vegetables (broccoli and cauliflower) indicating that irrigation water is a potential pre-harvest (in the field) source of bacterial contamination of vegetables.

Table 1: Main sources of pathogens or microbial contamination of leafy vegetables

Routes	Sources
On the farm	Contaminated irrigation water
	Animal waste fertilizers
	Wild and domestic animals
Off the farm	Post – harvest, washing in contaminated water
	Improper packaging
	Contamination from other foods in food preparation areas

(Modified from Berger *et al*., 2010)

Table 2: Sources of pathogenic microorganisms on fresh vegetables

Pre-harvest	Postharvest
Faeces	Faeces
Soil	Human handling (sorting, packaging, cutting, marketing including improper handling after wholesale or retail purchase)
Irrigation water	Washing
Green or inadequately composted manure	Storage
Human handling	

(Modified from Beuchat, 2006; Ijabadeniyi, 2010)

Bacteriological contaminants of some fresh vegetables irrigated with Awetu River water in Jima town, Southwestern Ethiopia, showed that the hygienic quality of both water and vegetables was poor due to the dominance of bacterial counts (6.09 to 7.10 $logCFUg^{-1}$ in vegetables and 7.42 $logCFUg^{-1}$ in water) (Weldezigna and Muleta, 2016). The examined microflora of the vegetables and water samples was dominated by *Bacillus* spp and enteric bacteria *S. aureus* and *Salmonella* spp which could cause foodborne diseases. Another study by Combarro *et al.* (1997) reported that different *Listeria* species were isolated from river water in Spain. River water when used for human and animal waste disposal poses a

health risk due to contamination with *Salmonella* spp and *Listeria* spp which could eventually contaminate vegetables if used for irrigation (Combarro *et al.,* 1997).

A comprehensive investigation of the quality of irrigation water used by farmers to irrigate indigenous leafy vegetables in urban and peri-urban areas of Morogoro including its bacteriological, quality is lacking. A component of this work investigated the bacteriological contaminants of common indigenous leafy vegetables irrigated by water from rivers and wells in urban and peri-urban areas of Morogoro, Tanzania.

Pathogens may also be transferred to the environment by application of inadequately composted or fresh animal manure (Santamaria and Toranzos, 2003). For example, a study conducted in southern Benin to assess the contamination of vegetables by fecal coliforms, (*E. coli* and fecal streptococci) following intensification of vegetable cropping through fertilization with poultry manure, demonstrated that the use of poultry manure as fertilizer during vegetable growing has influenced the fecal bacteria counts recorded on leafy eggplant, tomato, and carrot (Atidégla *et al*., 2016). Higher mean fecal bacterial counts ranging from 8 to 10 fecal coliforms, 5 to 8 fecal streptococci, and 2 to 6 *E. coli* were recorded. Another study carried out at the Department of Horticulture of the Food Engineering, College of the Federal University of Goiás, Brazil to evaluate microbiological quality of horticultural crops (*Lactuva sativa*), lettuce (*Raphanus sativus*), radish and (*Tetragonia expansa*), spinach, grown organically in the soil treated with bovine, chicken, and swine fresh manure, indicated that 63.3%, 50.0%, and 23.3% of the samples of lettuce, radish and spinach, respectively, contained $\geq 10^2$ total coliforms/g of product (Machado *et al*., 2006). In their study the presence of *E. coli* was confirmed in one sample of spinach, cultivated with cow manure. Johannessen (2005) noted that the possibly of lettuce to becoming contaminated with indicator organisms and pathogenic

bacteria from the use of animal manure was relatively small. However, the author recommended that more research is needed on possible transmission routes of pathogens in vegetable production.

The public health hazard of fresh vegetables contaminated with faeces, used as fertilizer, is a long-standing concern. In urban and peri-urban areas of Tanzania where demand for vegetables is increasing, there is lack of information in microbiological status of organic products (Jensen *et al*., 2013). There is, therefore, a need for scientific information on detection, survival, fate, and spread of pathogenic microorganisms in animal manure and vegetables cultivated in soils amended with animal manure.

2.4 Characteristics of Bacterial Species Isolated from Leafy Vegetables

Characterization of bacteria species isolated from leafy vegetables such as *Amaranthus hybridus* (Spinach), *Vernonia Moringa oleifera* (Horse radish), *Amagdalina* (Bitter leaf), *Lactuaca sativa* (Lettuce) and *Brassica oleracea* (Cabbage) have been done elsewhere by standard procedures involving morphological and biochemical procedures (Mackie and Mccartney, 1999; Aguoru *et al.,* 2015). Four species of bacteria common in leafy vegetables, and their association with manure and water mainly used for irrigation, have been reported including *Bacillus cereus*, *Escherichia coli*, *Listeria monocystogens* and *S. aureus* (Tambekar and Mundhada, 2006; Ijabadeniyi, 2010; Weldezgina *et al*., 2016).

A study conducted in Jaipur City, India on bacterial load isolated from Salads viz. carrot, coriander and cucumber applied the streak-plate method to obtain bacterial pure cultures on nutrient agar, MacConkey agar and Man Rogosa and Sharpe (MRS) agar from different bacterial isolates (Rajvanshi, 2010). The Isolates were characterized on the basis of their morphological appearance, biochemical and Gram's staining proportion. Isolates from all

the salad samples contained gram positive as well as gram negative bacteria. The gram positive bacteria include *Bacillus* spp, *Staphylococcus* spp *and Streptococcus* spp while gram negative were *E.coli*, *Pseudomonas* spp, *Enterobacter* spp, *Klebsiella* spp *and Citrobacter* spp.

Another study carried out in Ile-Ife, Southwestern Nigeria used nutrient agar, MacConkey agar, Eosine Methylene Blue agar, Blood agar and Mannitol Salt agar to characterise bacterial species isolated from Fluted Pumpkin Leaves (*T. occidentalis*) purchased from three different markets in the study area (Igbeneghu and Abdu, 2014). Morphological characteristics (colour, average colony-size, margin, surface elevation, opacity and consistency of colonies) and biochemical tests were used to identify the isolates to the presumptive species level based on standard test protocols. The identified isolates comprised Gram-negative organisms, which included *Proteus vulgaris*, *Enterobacter* spp., *Klebsiella* spp., *Serratia liquefaciens* and *Pseudomonas fluorescens* and Gram-positive organisms including *S. aureus* and *B. cereus.*

From the literature reviewed, information on bacterial characteristics from African indigenous leafy vegetables such as amaranth, nightshade and sweet potato leaves is inadequate or lacking. Procedures for identification of bacterial isolates from vegetables cultivated in urban and peri-urban areas in Tanzania and Morogoro in particular have not been documented. The current study characterized the bacterial isolates from three African indigenous leafy vegetables (amaranth, nightshade and sweet potatoes) using standard techniques including biochemical test (IMViC), micro and macro morphological analysis on nutrient agar, MacConkey agar, Blood agar, Mannitol Salt agar and Violet Red Bile agar with the aim to identify and document common bacteria in African indigenous leafy

vegetables and their associated sources i.e manure and water mainly used for irrigation in urban and peri-urban areas of Morogoro, Tanzania.

2.5 Antibiotic Sensitivity of Pathogenic Bacterial Isolates from Leafy Vegetables

Antibiotic resistance is generally referred to as the ability of bacteria and other microorganisms to resist the effects of an antibiotic. Resistance usually occurs when the drugs are used irrationally to treat a particular plant or animal (Brandl, 2006) or when bacteria acquire resistance genes from other closely related bacteria (Alonso *et al*., 2001). Antibiotic resistance is a growing problem worldwide and it poses a major threat to public and animal health (Caroline and Susan, 2013). High levels of antimicrobial resistance in humans are not only due to the abuse or indiscriminate use of antibiotics but also due to the presence of resistant organisms in herbs or foods eaten raw or minimally processed (McManus *et al*., 2002).

Antimicrobial susceptibility testing (AST) refers to in vitro methods used to determine the susceptibility of a bacterium to an antimicrobial agent (Buller *et al*., 2014).). The results assist clinicians to determine the most appropriate antimicrobial agents to treat infections (Morley *et al*., 2005). An important task of a clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates (Clinical and Laboratory Standards Institute, 2009). The goals of testing are to detect possible drug resistance in common pathogens and to determine their susceptibility to effective drugs of choice for particular infections.

Environmentally, antibiotic resistance can spread as the bacteria themselves move from place to place. For example, resistant bacteria can spread between animals and manure

used to amend the soils for crop production, water used for irrigation and bacteria in food which colonise the intestinal tracts of animals and humans (Fig. 1) (Tham *et al*., 2010; Rasheed *et al*., 2014). The use of large amounts of antibiotics in agriculture could lead to a selection of resistant bacteria. Also, applying manure from animal farms to agricultural fields or the use of contaminated water for irrigation could spread resistant bacteria to plants.

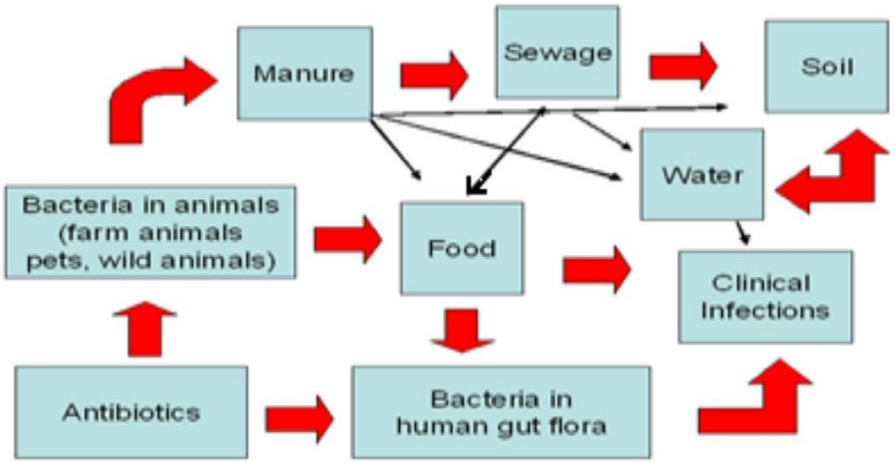


Figure 1: Antibiotic flow chart in bacteria and the environment.
(Source: Tham, 2012; Rasheed *et al.*, 2014).

High levels of antimicrobial resistance in humans are not only due to the abuse or indiscriminate use of antibiotics but also due to the presence of resistant organisms in herbs or foods eaten raw or minimally processed (Fig. 1) (McManus *et al*., 2002). The presence of antibiotic resistance both in normal flora and pathogenic microorganisms in fresh leafy vegetables may contribute to horizontal spreading of resistances between different isolates, species and genera.

Peter *et al*. (2005) showed that *Pseudomonas* spp, *Salmonella* spp and *Escherichia coli* could survive and grow in two of ten commercial insecticides, herbicides and formulations

used in the cultivation of leafy vegetables. For examples, 193 bacterial isolates from the leaves of *Teliferia occidentalis* samples tested for antimicrobial resistance in Nigeria, were found to be resistant to chloramphenicol, gentamicin, trimethoprim, ciprofloxacin and ofloxacin while 94% of the isolates were resistant to cephalothin as illustrated on the table below (Table 3) (Igbeneghu and Abdu, 2014).

Table 3: Distribution of Antibiotic Resistance among Isolated Bacterial Species in Nigeria

	No. of isolates resistant to antibiotics (%)					
Organisms (number isolated)	Ceph	Chlo	Gent	Trim	Cip	Ofl
Pr. vulgaris (48)	45 (94)	48 (100)	48 (100)	48 (100)	5 (10)	5 (10)
P. agglomeraus (35)	35 (100)	35 (100)	35 (100)	35 (100)	4 (11)	4 (11)
S. aureus (19)	19 (100)	16 (84)	19 (100)	19 (100)	8 (42)	10 (53)
E. aerogens (12)	12 (100)	12 (100)	10 (83)	10 (83)	1 (8)	1 (8)
S. epidermidis (11)	10 (91)	5 (46)	6 (55)	6 (55)	0 (0)	0 (0)
B. cereus (6)	5 (63)	5 (63)	8 (100)	8 (100)	2 (25)	2 (25)
B. subtilis (8)	5 (63)	5 (63)	5 (63)	8 (100)	2 (25)	2 (25)
P. fluorescens (5)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
S. liquefaciens (4)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)
K. pneumonia (4)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)
E. cloacae (3)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
B. sphearicus (2)	2 (100)	1 (50)	1 (50)	1 (50)	0 (0)	0(0)
K. oxytoca (1)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)
Unspeciated CoNS.(35)	35 (100)	35 (100)	35 (100)	30 (86)	12 (34)	16 (46)

Ceph= cephalothin, Chlo= chloramphenical, Gent= gentamicin, Trim= trimethoprim, Cip= ciprofloxacin and Ofl= ofloxacin; CoNS= Coagulase – negative Staphylococci (Source: Igbeneghu & Abdu, 2014).

A study conducted in India showed high percentages (about 20 %) of drug resistance for *E. coli* isolated from leafy vegetables and salads (Rasheed *et al.*, 2014). A gram-negative bacteria belonging to fecal coliforms, *Pseudomonas* spp, *Moraxella* spp, *Acinetobacter* spp, and *Flavobacterium-Cytophaga* groups, isolated from the rivers and bay water of Tillamook, Oregon, were reported to be resistant to chloramphenicol, streptomycin,

ampicillin, tetracycline, chlortetracycline, oxytetracycline, neomycin, nitrofurazone, nalidixic acid, kanamycin, and penicillin (Kelch and Lee, 1978). Over the years, it has been reported that resistance to cephalosporins among members of *Enterobacteriaceae* has increased mainly due to the spreading of Extended-spectrum ß-Lactamases. For example, Weldezgina and Muleta (2016) reported that *Staphylococcus aureus* and *Salmonella* spp isolates from fresh vegetables irrigated with Awetu River in Southern Ethiopia have shown high resistance to several selected antibiotics such as ampicillin, cefuroxime sodium, penicillin, tetracycline, erythromycin, cefuroxime sodium and penicillin. Other observations done by Threfall *et al*. (2000) suggest that resistance of these isolates could be of animal or human origin in which overuse or abuse of antibiotics create a selective pressure for antibiotic-resistant organisms.

A number of methods and corresponding guidelines to carry out antibiotic sensitivity of bacterial isolates exist worldwide for bacteria of human importance. Such methods include: the dilution method (broth and agar dilution method), disk diffusion method, e–test method, automated method and genotypic methods (PCR and DNA hybridization methods) (Jorgensen and Ferraro, 2009). The methods that have been used to determine the antibiotic sensitivity of bacterial isolates from vegetables and their associated sources are the dilution method and the disk diffusion method (Jorgensen and Turnidge, 2007). In this study the disc diffusion method was used to carry out antibiotic sensitivity of bacteria isolates from African indigenous leafy vegetables obtained from different sites in the Morogoro Municipality, Tanzania.

Little information is available on the occurrence of bacteria on the African indigenous leafy vegetables such as amaranth, nightshade and sweet potato leaves in urban and peri-urban areas in Tanzania. Data on the resistance or susceptibility of such contaminants to

commonly-used antibiotics such as enrofloxacin, rifampicin, sulphamethoxazole trimethoprim, ofloxacin, ampicillin, ciprofloxacin, gentamicin and streptomycin (Corpet, 1988) have not been documented adequately. This study was, therefore, undertaken to determine the antimicrobial resistance and sensitivity to commonly used antimicrobial agents (enrofloxacin, rifampicin, sulphamethoxazole trimethoprim and ofloxacin) in Morogoro, Tanzania. This study is intended to enforce measures to control the microbial contamination of foods, especially the African indigenous leafy vegetables.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Morogoro Municipality at Kichangani, Mafisa, Kingolwira and Tungi, wards where African leafy vegetables are cultivated in garden plots. Morogoro is located 200 km west of Dar es Salaam at the foot of Uluguru Mountains. The human population of Morogoro is estimated at 315 866 (NBS, 2012) with an average household size of 4.1. The selected areas have many smallholder farmers producing different African indigenous leafy vegetables. These farmers are the major suppliers of vegetables to vendors at the markets of Mawenzi and Morogoro Central market (recently demolished) and also to other smaller vegetable outlets.

3.2 Study Design

The study design was a cross sectional. Study units included; leafy vegetable smallholder garden plots from the selected areas, river and shallow well water, Mawenzi and Morogoro Urban Central markets. Three types of African indigenous leafy vegetables (amaranth, nightshade and sweet potato leaves) were collected. Fresh vegetables were collected directly from different garden plots as well as from Mawenzi and Morogoro Urban Central markets. Garden plots were selected randomly from all selected locations. Three samples of each vegetable were collected from the selected garden plots. Likewise, three samples of each of the vegetable types were collected from Mawenzi and Morogoro Urban Central markets. Water used for irrigation from the rivers and shallow wells were collected aseptically. Also, manure fertilised soil was sampled from the garden plots.

3.3 Sample Size

Since the population of bacterial load in African indigenous leafy vegetables in Morogoro Municipality is not known, the sample size was estimated using the equation 1 (Kothari, 2004).

$$n = Z^2.P(1 - P/d^2) \quad \text{..... (1)}$$

Where: n = sample size,

z = 95% confidence level (1.96),

P = probability proportional 50% and

d = acceptable error (0.05).

Hence, n= $(1.96)^2$.0.5(1-0.5)/(0.05) = 126. Therefore, a total of 126 samples were collected.

3.4 Data Collection and Sample Preparation

A total of 36 samples of the selected three indigenous vegetable types were collected from garden plots, while the other 18 fresh samples were purchased from the markets early in the morning and packed in sterile plastic bags as aseptically as possible. Also 27 samples of water, from three rivers, and nine (9) shallow well water samples as well as 36 samples of soil treated with manure were collected in sterile containers. All samples collected (n = 126) were immediately transported to the Microbiology Laboratory of the Pest Management Centre, Sokoine University of Agriculture (SUA), Morogoro, for bacteriological analysis. Biochemical tests were done in the Microbiology Laboratory of the College of Veterinary and Medical Sciences. Damaged and diseased parts of the leafy vegetables were removed. All laboratory tools for the preparation of cultures such as chopping boards, knifes and vegetable trays were sterilized by autoclaving at 121°C for 15

min. Samples of 25g were chopped aseptically from each sample into approximately 1 to 2 cm pieces and vigorously shaken in 250 ml of sterile normal saline water for 3 min to homogenize the samples. The wash fluids of the vegetable samples were serially diluted 5-fold and analysed for total bacterial count. A total of 25 g of soil samples fertilized with manure were mixed with 250 ml sterile normal saline to prepare an emulsion which was also serially 5-fold diluted and analysed for bacterial count.

3.5 Total Bacterial Count

Total bacterial count of the wash fluid of leafy vegetables, soil emulsion and the irrigation water samples were determined using the plate count technique and expressed as colony forming unit (CFU/ml). For the enumeration of microorganisms present in each sample, a serial dilution of each sample was carried out and 0.1 ml of 10^{-3}, dilution factor were inoculated in nutrient agar and then incubated aerobically at 37ºC for 24 hrs. Thereafter, the plates were removed and the colonies counted. The dilutions which produced countable number of bacteria of between 30 and 300 colonies were selected for further identification of the bacteria species as proposed by Jolt *et al*. (1994).

3.6 Identification of Bacterial Isolates from Leafy Vegetables Washings, Irrigation Water and Soil Emulsion

Colonies of interest were picked from the nutrient agar and sub-cultured in Blood agar, MacConkey agar and Violet red bile agar and incubated at 37ºC for 24 hours to obtain pure cultures. The macro – morphology of bacterial isolates from pure cultures of Blood agar, MacConkey and Violet red bile agar were aerobically assessed for colony morphology, and further studied through gram staining and standard biochemical tests as discussed by Tsado *et al.* (2013). The gram positive bacteria presented with purple colour after being stained with crystal violet and decolorized with ethanol and acetone while

gram negative appeared red or pink colour (Taura and Habibu, 2009). Standard biochemical tests of the different bacterial isolates were carried out. These consisted of Indole Production, Methyl red, Voges Proskauer, and Citrate (IMViC), catalase production and urease production as documented by Nwachukwu and Chukwu, (2013). IMViC and urease tests were used to characterize some enteric bacteria.

For the IMViC test, the bacteria were inoculated on blood agar, MacConkey agar and Violet Red Bile agar and incubated for 24 hrs. Inoculation of a bacteria colony from agar (blood agar, MacConkey and Violet Red Bile) was done in the IMViC scale vials, allowed to stand for 2 to 3 min then incubated for 24 hrs at 37^0C. The results were interpreted by examining the colouration in the IMViC scale vials as outlined hereunder:

i. For indole test, a few drops of Kovac's reagent (The Hardy Diagnostics manufacturing Facility and Quality Management System, 2016) were added
ii. For Methyl Red test, methyl red (MR) – reagent was added
iii. For Voges Proskauer, KOH was added followed by α – naphptol

As for indole and methyl red test the predictable colour for positive result is red (MacFaddin, 2000), while for Voges Proskauer the indication for positive result is pink colour (Isenberg, 2007). In citrate test the appearances of blue colour would indicate positive results, after inoculation and incubation of the bacteria colony for 24 hrs (Jorgensen *et al*., 2015).

Catalase is an iron containing enzyme which catalyses' the decomposition of H_2O_2. This tests help to confirm *Staphylococcus* spp when colonies are subjected to hydrogen peroxide (Weldezgina and Muleta, 2016). A sterilized wire loop was used to pick a colony of bacteria unto a slide followed by a drop of H_2O_2. Where formation of gas bubbles

occurred, it was an indication of catalase positive results (Taura and Habibu, 2009). Urease test was used to detect *Proteus* spp, which secrete the urease enzyme to catalyse the conversion of urea to ammonia. Ammonia raises the PH of the medium and changes the colour of the test to red if urease was produced by the isolate (ASM Microbe Library, 2015).

3.7 Antibiotic Sensitivity Testing

The isolates sensitivity or resistances to antibiotics were determined on Muller – Hinton agar plates by the disk diffusion technique (Bauer *et al*., 1966). The antibiotic discs were placed on the medium by using a sterilized forceps and incubated at 37^0C for 24 hrs. Results were recorded as resistant or sensitive based on absence or presence of a zone of inhibition as described by Fouad (2011). The zones of inhibition were measured manually with a transparent ruler at one mm accuracy. The antibiotics that were used in this study included: enrofloxacin (5 mg), rifampicin (5 mg), ofloxacin (30 mg) and sulphamethoxazo-trimethoprim (25 mg), based on the recommendation of standard treatment guidelines (STG) and the National Essential Medicines list (NEMLIT) of Tanzania (Ministry of Health, Gender, Community Development, Eldery and Children, 2007). The common antibiotics e.g gentamicin and ampicillin were not used because the selected antibiotics were readily available and are capable of testing for both positive and negative bacteria (CLSI, 2017).

3.8 Data Analysis

Bacterial counts were calculated as colony forming units per millilitre of washings and soil emulsion (CFU/ml). Data was subjected to Analysis of Variance (ANOVA) using SAS statistical software. Means were separated by Least Significant Difference (LSD) to test significant differences (at 5 % level of probability) on bacterial load among different

treatments and locations. Descriptive statistical analysis was employed to explore the relationship between bacterial loads of the different leafy vegetables, by location, source and their corresponding antibiotics sensitivity or resistance.

3.9 Ethical Consideration

Research permit was provided by the Vice Chancellor, Sokoine University of Agriculture, Morogoro, Tanzania and permission letters were obtained from Morogoro Regional Administrative Secretary and Municipal Councils of Morogoro Urban. Verbal consent was obtained from vegetable farming community and each vendor of the vegetables after explaining the purpose and importance of the study prior to commencement of sampling. All the information collected from the study sites and the laboratory results obtained after analysis were kept under the custody of the researcher as confidential.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Bacterial Load of Selected African Indigenous Leafy Vegetables Cultivated in Different Farm Sites and Market Outlets

4.1.1 Bacterial load from leafy vegetables at different farm sites

Bacterial loads from the three African indigenous leafy vegetables (amaranth, nightshade and sweet potato leaves) cultivated at farm sites in Morogoro Municipality are presented in Table 4. The results show the mean number of bacteria per ml of the samples in CFU. The bacterial loads from the studied vegetables were in the range of 10^5 CFU/ml. These high counts could be attributed to the unhygienic practices at the farms. Generally, the number and type of microorganisms found on fresh produces were highly variable. The commonly reported mesophilic bacteria counts were in the range of 10^3–10^9 CFU/ml in raw vegetables after harvest, depending on the produce and the growing conditions (Zagory, 1999; Oliveira *et al*., 2010). The International Commission on Microbiological Specifications for Food (ICMSF) has set the standard limit of 4.9×10^6 aerobic count/g for wet weight vegetables (ICMSF, 1998; Benti *et al*., 2014).

According to FAO (1979), the standard limit for aerobic mesophilic bacterial count for food should be less than 10^5 CFU/ml. Although, the results of this study indicate the number of microbial load to be within the standard bacterial count recommended by WHO, FAO/WHO (2008), vegetables produced and consumed within the study area can still pose a great health risk to consumers.

Table 4: Bacterial load from African indigenous leafy vegetables at different farm sites

Vegetable type	Farm sites	Average bacterial load (CFU/ml)	SD	n
Amaranth	Tungi	1.8×10^5	O.22	3
	Kichangani	1.7×10^5	0.25	3
	Kingolwira	1.8×10^5	0.78	3
	Mafisa	1.6×10^5	0.50	3
Nightshade	Tungi	1.7×10^5	0.14	3
	kichangani	1.9×10^5	0.23	3
	Kingolwira	1.7×10^5	0.68	3
	Mafisa	1.9×10^5	0.22	3
Sweet potato leaves	Tungi	1.5×10^5	0.44	3
	kichangani	1.6×10^5	0.75	3
	Kingolwira	1.6×10^5	0.88	3
	Mafisa	1.4×10^5	0.70	3

CFU = Colony Forming Unit, SD = Standard Deviation, n = Number of Observation

Sweet potato leaves registered a lower bacterial load (1.5×10^5 CFU/ml) than amaranth (1.7×10^5 CFU/ml) and nightshade (1.8×10^5 CFU/ml) in all the studied sites though not significantly different. Nightshade showed a relatively higher number of bacteria load when compared to the other indigenous leafy vegetables i.e. amaranth and sweet potato leaves but not significantly different. From the results, the isolated bacterial loads of African indigenous leafy vegetables studied at the different farm sites in the study area were in the order of magnitude nightshade > amaranth > sweet potato leaves. The loads were, however, not significantly different. The results obtained have shown that microorganisms are abundant on the surface of vegetables. According to Frank-Peterside and Waribor (2006), bacterial load on leafy vegetables increases with time during storage. If the counts of these are high then they can pose dangers to consumers.

4.1.2 Bacterial load of African indigenous leafy vegetables in different market outlets

Bacterial loads isolated from the three African indigenous leafy vegetables (amaranth, nightshade and sweet potato leaves) sold at market outlets in Morogoro are presented in Table 5. The results of this study indicate that the investigated vegetables sold in the Morogoro market outlets contained microbial loads ranging from 1.2×10^5 to 1.8×10^5 CFU/ml (Table 5). In this study, nightshade had the highest bacterial count of 1.8×10^5 CFU/ml and amaranth had the lowest bacterial count of 1.2×10^5 CFU/ml all sold at Morogoro Central Market (Table 5). Vegetables sold in Mawenzi market registered the lowest bacterial count (1.2×10^5 CFU/ml). The higher bacterial counts observed for nightshade and sweet potato leaves in the Central urban market outlet could be attributed to unhygienic handling during processing and marketing of the vegetables (Plate 1). The works of Uze *et al.* (2009) reported higher bacterial counts ranging from 10^8 to 10^9 CFU/ml for vegetables consumed fresh and commonly sold in Nigerian Market outlets.

Table 5: Bacterial load isolated from African indigenous leafy vegetables sold at different market outlets

Vegetable type	Market outlets	Average bacterial load CFU/ml	SD	n
Amaranth	Central urban market	1.2×10^5	0.05	3
	Mawenzi market	1.2×10^5	0.04	3
Nightshade	Central urban market	1.8×10^5	0.30	3
	Mawenzi market	1.2×10^5	0.03	3
Sweet potato leaves	Central urban market	1.7×10^5	0.08	3
	Mawenzi market	1.2×10^5	0.05	3

CFU = Colony Forming Unit, SD = Standard Deviation, n = Number of Observation

Plate 1: Handling, processing and marketing of African indigenous leafy vegetables at Central Urban market outlet in Morogoro Municipality, Tanzania

Results show that nightshade significantly (P = 0.003) registered a higher bacterial load 1.5×10^5 cfu/ml (Table 6). Likewise the bacterial load of amaranth was significantly lower (1.2×10^5 CFU/ml) (P = 0.004) than that of nightshade (1.5×10^5 CFU/ml) and sweet potato leaves (1.4×10^5 CFU/ml) but there was no significant difference between the bacterial load of nightshade and sweet potato leaves (Tables 6). Generally, the bacterial loads from African indigenous leafy vegetables sold in different market outlets of Morogoro Municipality, showed the trend nightshade > sweet potato leaves >amaranth (Table 6). As reported by Isa *et al*. (2014), high bacterial count on vegetables sold in market outlets largely depend on the produce, the growing conditions and handling during processing and selling of the product (Plate 1). Generally, vegetables are exposed to microbial contamination at every step including: cultivation, harvesting, transporting, packaging, storage (Nyanteng, 1998) and selling to the consumers (Nipa *et al.*, 2011) (Plate 1). Therefore, there is need to protect the health of the consumers by proper washing and disinfection of these products, which in most cases are consumed raw as salads or semi prepared snacks.

Table 6: Mean separation of bacterial load isolated from African indigenous leafy vegetables sold at different market outlets

Vegetable types	Average bacterial load (CFU/ml)
Amaranth	1.2×10^5b
Nightshade	1.5×10^5a
Sweet potato leaves	1.4×10^5a
$LSD_{0.05}$	**0.2×10^5**

Letters 'a' and 'b' indicate the level of significance; the mean values followed by the same letter(s) do not differ significantly at $P < 0.05$

4.2 Major Sources of the Microorganisms Found on Leafy Vegetables in the Study Area

Bacterial loads of river water used for irrigation by most farmers for the three African indigenous leafy vegetables at different farm sites in the Morogoro Municipality ranged from 1.6×10^5 CFU/ml to 1.8×10^5 CFU/ml (Table 7). Bacterial counts recorded in the river water from different sections (upper, middle and lower) varied from 0.6×10^5 to 2.5×10^5 CFU/ml (Table 6). The mean bacterial count of water samples from lower river sections ranged between 1.6×10^5 and 2.5×10^5 CFU/ml compared to $\leq 1.7 \times 10^5$ CFU/ml in the middle and upper sections of the river (Table 7). Generally, the results of bacterial load of the lower sections of the river at different farm sites showed the trend Tungi (2.5×10^5 CFU/ml) > Kichangani (1.8×10^5 CFU/ml) > Kingolwira (1.6×10^5 CFU/ml) (Table 7).

In the study area, vegetables are predominantly cultivated in the lower slopes of the landscape where the terrain is flat and fertile due to river depositions of fine clay soils and organic materials eroded from higher slopes (Plate 2) (Msanya *et al*., 2003). The recommended maximum total coliform and heterotrophic bacterial counts limit in river water for no risk in South Africa is 5 CFU/100 ml or 5×10^2 CFU/ml (WRC, 1998). The bacterial loads of the river irrigation water in this study were in the range of 10^5 CFU/ml.

As discussed in section 4.3 of this study, bacterial species including *E. coli*, *Enterobacter aerogenes, and Proteus* spp (coliform bacteria), *Staphylococcus albus* and *Bacillus* spp were isolated from the studied vegetables and soils treated with manure. These types of bacteria were either coliforms and/or heterotrophs.

Table 7: Bacterial load isolated from river irrigation water at different farm sites

Source type	Farm sites	Position	Average bacterial load CFU/ml	SD	n
River irrigation water	Tungi	Lower	2.5×10^5	0.32	3
		Middle	1.7×10^5	0.72	3
		Upper	0.8×10^5	0.34	3
	Kichangani	Lower	1.8×10^5	0.30	3
		Middle	1.4×10^5	0.97	3
		Upper	0.6×10^5	0.32	3
	Kingolwira	Lower	1.6×10^5	0.97	3
		Middle	0.8×10^5	0.41	3
		Upper	0.7×10^5	0.20	3

CFU = Colony Forming Unit; SD = Standard Deviation; n = Number of Observation

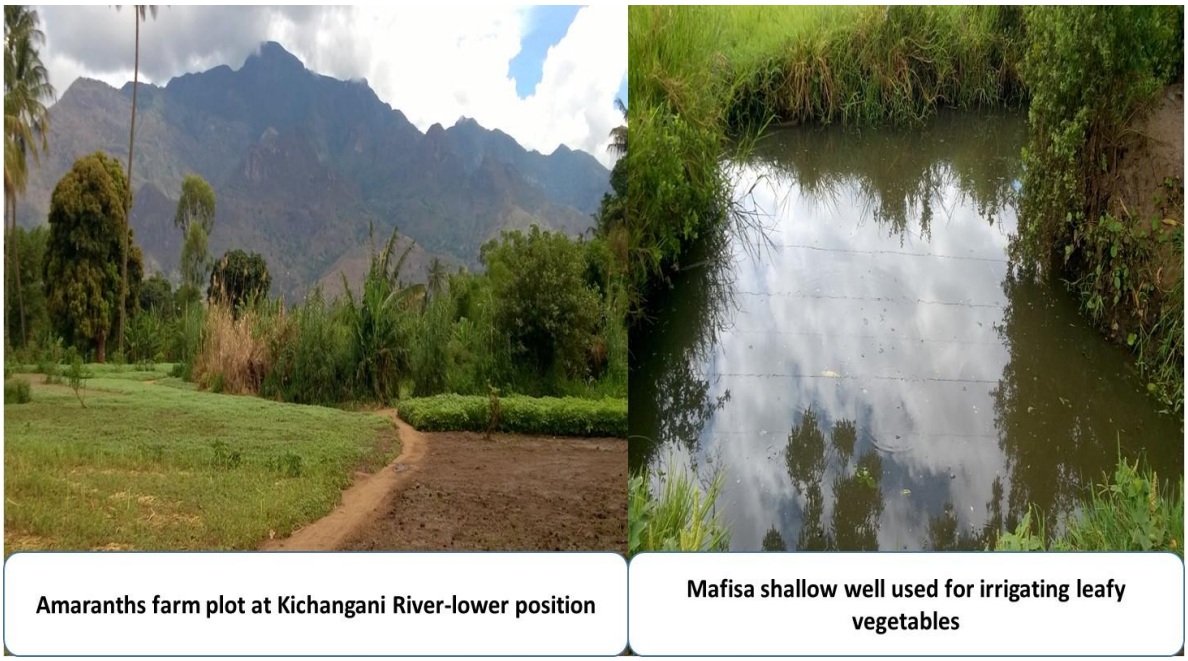


Plate 2: African indigenous leafy vegetables cultivated in the lower slopes of the landscape and irrigation water from shallow well in the study area

The bacterial loads of the river water used for irrigating vegetables in the study area raise both environmental and public health concerns. Possible sources of contamination of the river water include erosion and flooding, human and animal faeces or introduction of micro-organisms by birds and insects (Paul *et al*., 1995; Nevondo and Cloete, 1999). Most of the river sources are reportedly prone to higher bacterial contamination levels due to heightened ecological activities such as farming practices (Obi *et al*., 1998). The results show further that river irrigation water drawn from lower sections of the river at Tungi, Kichangani and Kingolwira farm sites registered significantly higher bacterial loads with an average of 1.8×10^5 CFU/ml compared to the middle (1.3×10^5 CFU/ml) and upper (0.8×10^5 CFU/ml) sections respectively (Table 8).

At Mafisa farm site, there is no river crossing the area and hence farmers irrigate their vegetables using water drilled from shallow wells (Plate 3). Bacterial loads of the well irrigation water at Mafisa farm site were lower ($<1.4 \times 10^5$ CFU/ml) (Table 9) when compared to bacterial loads of the lower sections of the river irrigation water at the other sites (Table 8), but still higher than the allowed maximum total coliform and heterotrophic bacterial counts in river water for no risk in South Africa (WRC, 1998).

Table 8: Mean separation of bacterial load isolated from river irrigation water

Source type	Section	Average number of bacterial load (CFU/ml)
River Irrigation water	Lower	1.8×10^5a
	Middle	1.3×10^5ab
	Upper	0.8×10^5ab
$LSD_{0.05}$		**0.5×10^5**

Table 9: Bacterial load isolated from well irrigation water at Mafisa farm site

Source type	Farm sites	Section	Average number bacterial load (CFU/ml)	SD	n
Well water	Mafisa	Well one	1.2×10^5	0.82	3
		Well two	1.4×10^5	0.36	3
		Well three	1.3×10^5	0.57	3

SD = Standard Deviation, n = Number of Observation, CFU = Colony Forming Unit

Bacterial loads of soil treated with manure to fertilise the leafy vegetable plots in Morogoro Municipality ranged between 1.5×10^5 and 2.2×10^5 CFU/ml) (Table 10). These results relatively correlate with the previously reported bacterial counts for soils treated with manure elsewhere (Edesi *et al*., 2013; Obi *et al*., 2014). Obi *et al*. (2014) reported overall higher total bacterial count, ranging from 5.9×10^4 CFU/ml to 1.75×10^9 CFU/ml for soils treated with manure in Nigeria.

Total bacterial count analysed for different types of manure treated soils, including organic with green and cattle manure, organic with green manure and conventional with green and cattle manure conducted in a field trial in Central- Estonia ranged from 5.78 x 10^6 to 9.75 x 10^6 CUF/g (Edesi *et al*., 2013). The relatively high total bacterial counts, detected among the soil samples surveyed in this investigation support contamination of the vegetables with pathogenic microorganisms which may present a potential health hazard to consumers.

Total bacterial loads did not show significant difference among the soils of different farm sites treated with manure. Studies conducted elsewhere show that there are numerous factors that may influence the survival of bacteria in the soil. Such factors include: moisture, soil type, manure application rate, nutrient availability, temperature and pH (Entry *et al*., 2000; Mubiru *et al*., 2000 and Jamieson *et al*., 2002).

Table 10: Bacterial load of soil manure at different farm site

Source type	Farm site	Position	Average bacterial load CFU/ml	SD	n
Soil manure	Tungi	plot one	2.1×10^5	0.15	3
		plot two	2.1×10^5	0.62	3
		plot three	1.8×10^5	0.24	3
	Kichangani	plot one	1.7×10^5	0.66	3
		plot two	1.3×10^5	0.83	3
		plot three	1.7×10^5	0.25	3
	Kingolwira	plot one	1.6×10^5	0.51	3
		plot two	2.2×10^5	0.55	3
		plot three	2.0×10^5	0.12	3
	Mafisa	plot one	1.8×10^5	0.50	3
		plot two	1.6×10^5	0.56	3
		plot three	1.5×10^5	0.30	3

SD = Standard Deviation, n = Number of Observation, CFU = Colony Forming Unit

4.3 Characteristics and Frequency of the Bacterial Species Isolated from African Indigenous Leafy Vegetables at Farm Sites, Market Outlets and Other Sources

4.3.1 Major characteristics of bacterial species

The major characteristics of the bacterial species isolated from African indigenous leafy vegetables at farm sites, market outlets, irrigating waters and soil manure are presented in (Table 11, Plates 3 and 4). The isolated bacterial species include: *E. coli, E. aerogenes* and *Proteus* spp, (coliforms and enteric bacteria), as well as *S. albus*, and *Bacillus* spp (Table 11). These results are similar to those of previous studies on bacterial species isolated from fresh vegetables and other food products elsewhere. (Oluwafemi and Semisaye, 2005; Odu and Akano, 2012). Other characteristics that were used to identify the studied bacterial species include the biochemical tests.

Table 11: Occurrence and characteristics of bacterial isolates from selected African indigenous leafy vegetable and sources at different farm sites and market outlets

Sample type	Bacterial species isolated	Colony characteristic/ gram reaction	Biochemical test					
			ID	MR	VP	CT	CA	UR
Amaranth Sweet potato leaves Nightshade	*Escherichia coli*	**BA**: white, smooth and non-haemolytic **MC**: lactose ferment, flat and dry. **VRB**: pinkish red and dry Gram –ve, rods	+	+	-	-	NT	NT
Amaranth Nightshade Sweet potato leaves River water Well water	*Enterobacter aerogenes*	**BA**: white, medium and non-haemolytic **MC:** late lactose ferment, slight mucoid **VRB:** pink in colour and medium Gram –ve, short rods and bipolar stain	-	-	+	+	NT	NT
Amaranth Nightshade Sweet potato leaves River water	*Proteus* spp	**BA:** opaque, circular and non-haemolytic **MC:** non-lactose ferment and colourless **VRB:** no growth Gram –ve, rods	-	+	-	+	NT	+
Amaranth Nightshade Sweet potato leaves Soil treated with manure	*Staphylococcus albus*	**BA:** yellow, raised and non-haemolytic **MC:** no growth **VRB:** no growth Gram +ve, cocci and mostly in singly and pair	NT	NT	NT	NT	+	NT
Soil treated with manure	*Bacillus* spp	**BA:** green, large, flat, hairy and spreading colonies and β-haemolytic **MC:** no growth **VRB:** no growth Gram +ve, large rods round end and forming endospore	NT	NT	NT	NT	NT	NT

BA = Blood agar, MC = MacConkey, VRB = Violet Red Bile agar, ID = Indole chemical test, MR = Methyl red chemical test, VP = Voges proskeur, CT = Citrate, CA = Catalase, UR = Urease, NT = Not Tested, + = Detected, - = Not detected.

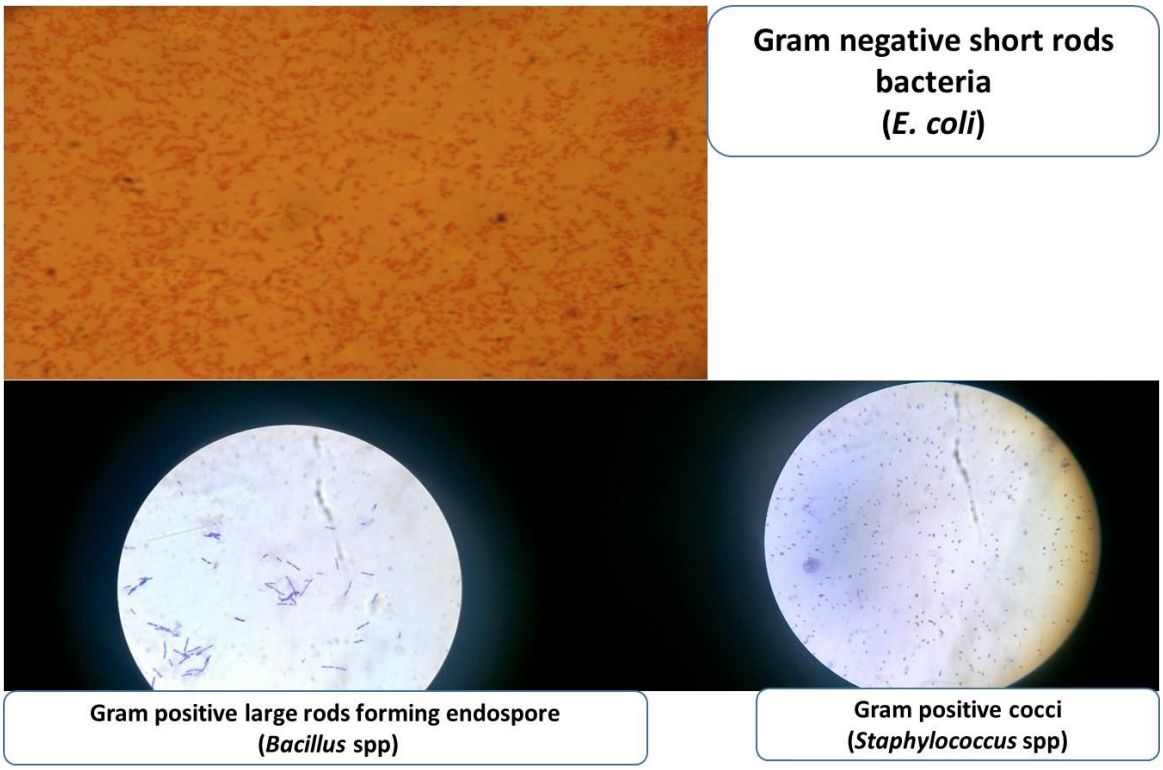


Plate 3: Micro-morphological characteristics for bacterial identification

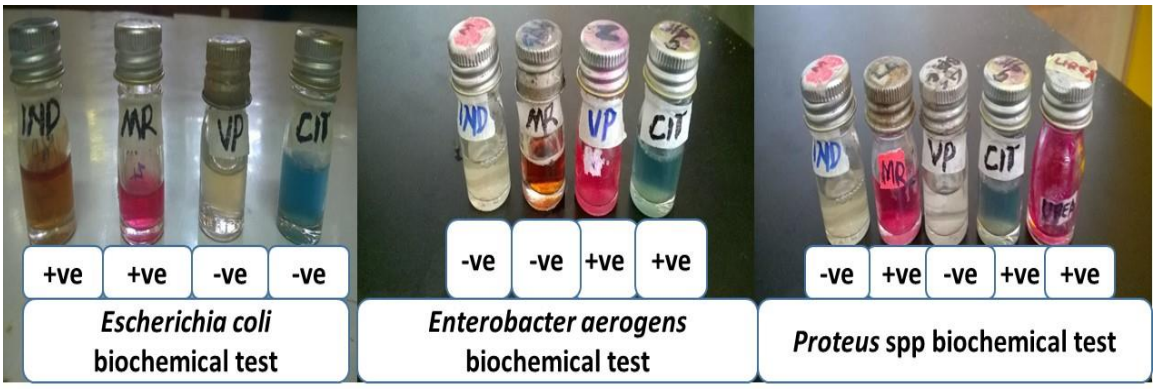


Plate 4: Characteristics of the bacterial species isolated from the leafy vegetables by biochemical tests (Indole (IND), Methyl red (MR), Voges Proskeur (VP), Citrate (CIT) and Urease)

In this study *E. coli* was positive for indole and methyl red tests and negative for Voges Proskeur and citrate tests whereas *E. aerogenes* was negative in both indole and methyl red tests, and positive in Voges Proskauer and citrate tests (Table 11). *Proteus* spp was negative for indole test and Voges Proskeur and positive for methyl red citrate and urease tests, while *Staphylococcus albus* was positive for catalase production. No colour appearance was observed for *Bacillus* spp in the biochemical tests.

The results of this study show that *E. coli* was identified in all the studied vegetables, while *E. aerogenes* was detected in all the studied vegetables and irrigation water. *S. albus* dominated in all the vegetable samples and the soil treated with manure. *Bacillus* spp was detected only in the soil treated with manure (Table 11). These results suggest that bacterial species are not uniformly distributed in the different vegetable types and sources, probably due to numerous factors that may influence the survival of bacteria in the fresh produce and in soil such as moisture, soil type, manure application rate, farming practices, nutrient availability, temperature and pH (Entry *et al.*, 2000; Mubiru *et al.*, 2000 and Jamieson *et al.*, 2002).

4.3.2 Occurrence of bacterial isolates

The frequency of *E. coli* bacteria isolated from African indigenous leafy vegetables collected at different farm sites and market outlets is presented in Fig. 2. High frequency of *E. coli* was observed in amaranth and sweet potato leaves than in nightshade. However, the frequency of *E.coli* in amaranth and sweet potato leaves was relatively lower in market outlets than in the farm sites (Fig. 2. The observed results could be attributed to borehole water commonly used for irrigating and washing the vegetables at farm plots and market outlets respectively.

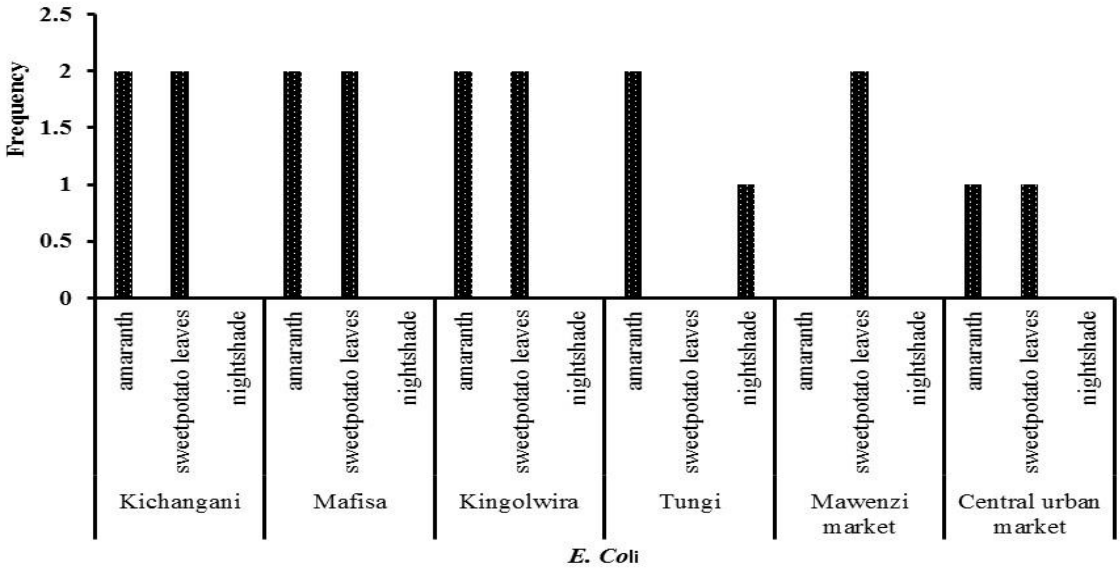


Figure 2: Frequency of *E. coli* from African indigenous leafy vegetables at the farm sites and market outlets

A study conducted in Tanzania's commercial capital, Dar es Salaam has found that majority of boreholes and the piped water were contaminated with bacteria (*E. coli*) found in human and/or animal feces (Kihupi *et al*.; 2016). The presence of *E. coli* in the vegetables could therefore suggest faecal contamination. *E. coli* is part of the normal flora of the human and animal intestines (Hassan *et al.,* 2006). Some strains of *E. coli* have been linked to diarrhoea, gastroenteritis and urinary tract infections (Aguoru *et al.,* 2015).

The frequency of *Enterobacter aerogenes*, was high in the nightshade than in sweet potato leaves and amaranth at Kichangani and Mafisa farm sites (Fig. 3). At Kingolwira and Tungi farm sites, the frequency of *E. aerogenes* from the same vegetables was observed to be low (Fig. 3). From the market outlet, nightshade seems to have had a high frequency of *E. aerogenes* compared to the other vegetables (Fig. 3).

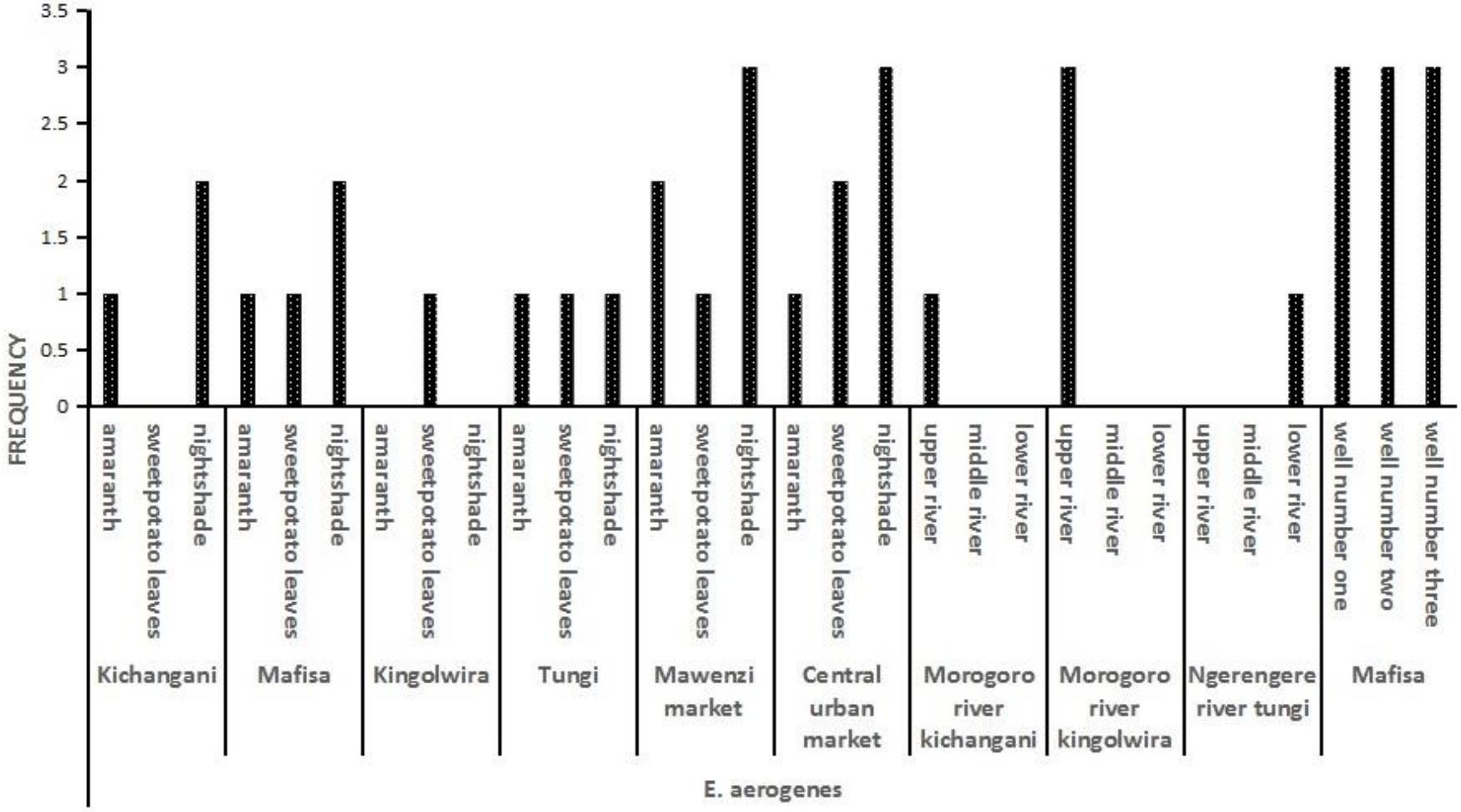


Figure 3: **Frequency of *Enterobacter aerogenes* from different African indigenous leafy vegetables and water sources**

Frequency of *E. aerogenes* was higher in the upper river at Kingolwira compared to Kichangani and Tungi. The well water source at Mafisa indicated similar frequency of *E. aerogenes* (Fig. 3). *E. aerogenes* is known to cause opportunistic infections, meaning that it will usually cause a disease only in a person or host that has a compromised immune system (Falomir *et al.*, 2010). Studies are now showing increased alarm in community infections (Odu and Akano, 2012).

Proteus spp had high frequency in the nightshade vegetables at Kingolwira farm site than in the amaranth and sweet potato leaves (Fig. 4). Medium frequency of *Proteus* bacteria was also observed in the river water, especially in the middle and lower sections. *Proteus* spp. bacteria present in soil or water habitats are often regarded as indicators of fecal pollution, posing a threat of poisoning when the contaminated water or seafood is consumed (Drzewieckal, 2016). Contamination of the river water in the middle and lower sections could be explained by erosion and flooding, and human and animal faeces deposited on these river sections due to reduced river flow gradient (Nevondo and Cloete, 1999). Medium frequency of *S. albus* was observed in nightshade and amaranth at Tungi farm site and central urban market outlet (Fig. 5). High frequencies were also observed in the soil treated with manure at Kichangani farm site (Fig. 5). *Proteus* spp are known to cause serious respiratory tract infection among immuno compromised individuals (Jawetz *et al*., 2013; Uzeh *et al.*, 2009).

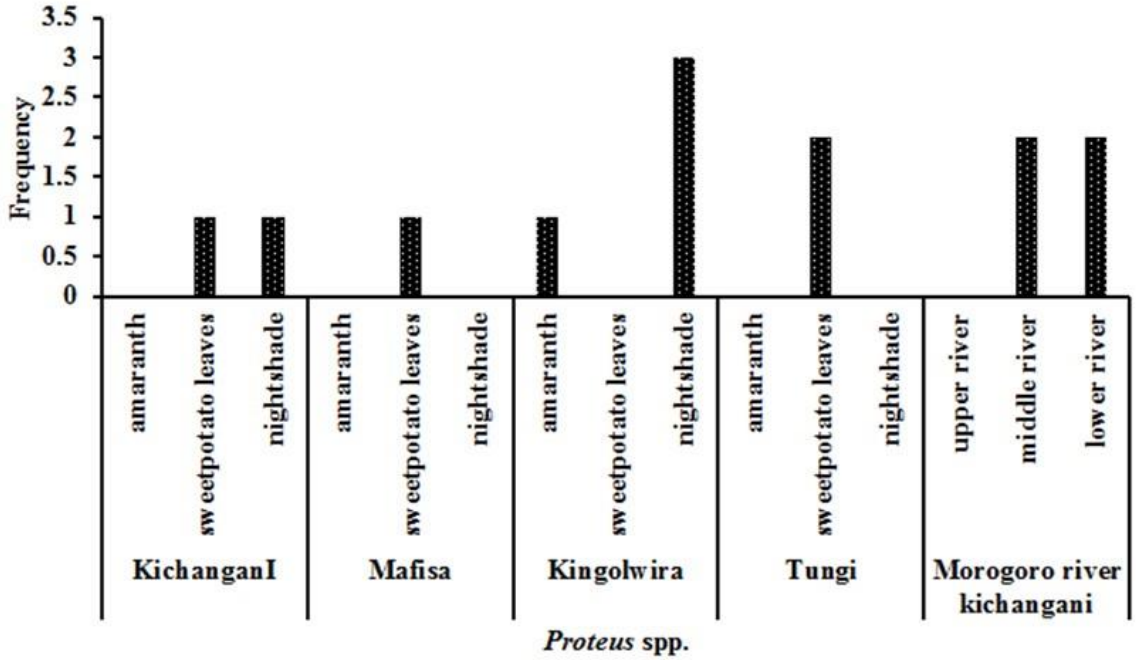


Figure 4: **Frequency of *Proteus* spp from different African indigenous Leafy vegetables and water sources**

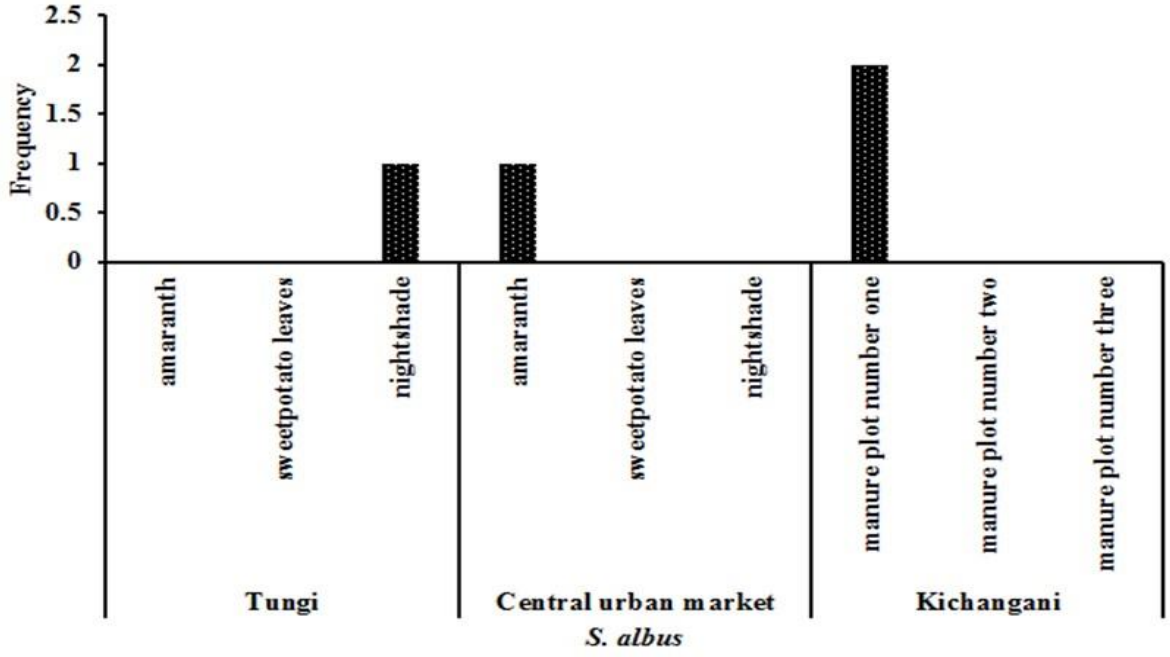


Figure 5: **Frequency of *Staphylococcus albus* from different African indigenous Leafy vegetables and manure sources**

The presence of *Staphylococcus* spp in high frequency in some vegetables (nightshade and amaranths) and in the soil treated with manure suggests potential health risks because of its ability to cause a wide variety of infectious diseases. This bacterium has been implicated to cause food poisoning, cellulitis and toxic shock syndrome (Medicine Net, 2015). According to the United State Food and Drug Administration (USFDA, 2015), the presence of this bacterium or its enterotoxins in processed foods or on food processing equipment is an indication of poor sanitation. The agency emphasized that *Staphylococcus* spp has been identified as the causative agent in many food poisoning outbreaks in many parts of the world.

The frequency of *Bacillus* spp in soil treated with manure from the investigated farm sites show similar trend. This bacterium was not detected in any of the three African indigenous leafy vegetables (amaranth, nightshade and sweet potato leaves) or in the water sources (well water and river water) used for irrigation. Literature show that *Bacillus* spp are widespread in nature and are frequently isolated from soil and growing plants (Stenfors Arnesen *et al*., 2008). The *Bacillus* spp are known to cause food borne diseases such as the emetic and diarrhoea syndrome due to the production of enterotoxins (Valero *et al*., 2002).

4.4 Antibiotics Sensitivity of Potentially Pathogenic Bacterial Isolates From African Indigenous Leafy Vegetables at Farm Sites, Market Outlets and in other Sources

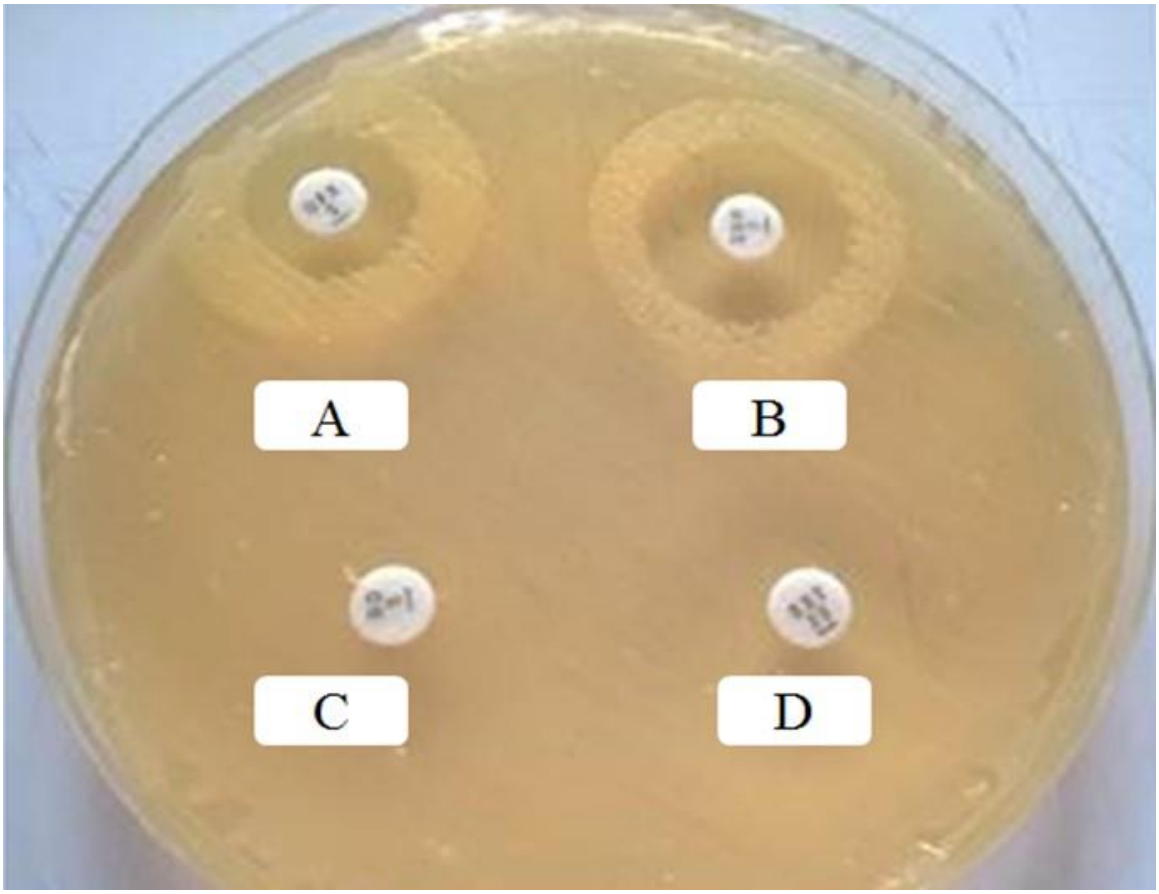
In this study *E. coli*, *E. aerogenes* and *Proteus* spp, *S. albus*, and *Bacillus* spp were isolated and biochemically confirmed (Table 11). These bacteria were isolated from a total of 126 samples of African indigenous leafy vegetables, (amaranth, sweet potato leaves, nightshade), river and well-water used for irrigation and soil treated with manure. The antibiotic sensitivity of five isolates against four different antibiotics are presented in

Table 12 and Plate 5. The results indicate *E. coli* isolates were most susceptible to enrofloxacin, sulphamethoxazole and ofloxacin.

Table 12: Sensitivity pattern of *E. coli, E. aerogenes* and *Proteus* spp isolated from different leafy vegetable types and sources using disc diffusion method

Antimicrobial agent	*Escherichia coli*			*Enterobacter aerogenes*			*Proteus* spp		
	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)
Enrofloxacin R = ≤ 16 I = 17 – 20 S = ≥ 21	5(27.8)	0(0.00)	13(72.2)	8(21.6)	0(0.00)	29(78.3)	1(8.3)	0(0.00)	11(91.7)
Sulphameth - oxazole R = ≤ 10 I = 11 – 15 S = ≥ 16	11(61.1)	0(0.00)	7(38.9)	6(16.2)	0(0.00)	31(83.8)	3(25)	0(0.00)	9(75)
Ofloxacin R = ≤ 12 I = 13 – 15 S = ≥ 16	6(33.3)	0(0.00)	12(66.7)	9(24.3)	0(0.00)	28(75.7)	2(16.7)	0(0.00)	10(83.3)
Rifampicin R = ≤ 16 I = 17 -19 S = ≥ 20	0(0.00)	18(100)	0(0.00)	0(0.00)	37(100)	0(0.00)	0(0.00)	12(100)	0(0.00)

I = Intermediate, R = Resistant and S = Sensitive



Ofloxacin (A) and enrofloxacin (B) demonstrated sensitivity while rifampicin (C) and sulphamethoxazole (D) were resistant

Plate 5: Antibiotic resistance and sensitivity of bacterial isolates determined by the absence or presence of a zone of inhibition on Muller – Hinton agar plates

The total of the 18 isolated *E. coli*, 13 (72.2%) were susceptible to enrofloxacin and five (27.8%) showed intermediate characteristics, while twelve (66.7%) were susceptible to ofloxacin and six (33.3%) were intermediate (Table. 12). As for sulphamethoxazole, seven (38.9%) *E. coli* isolates were susceptible and 11 (61.1%) were intermediate. None of the *E. coli* was resistant to enrofloxacin, sulphamethoxazole and ofloxacin, but all were resistant to rifampicin (Table 12).

Out of 37 *Enterobacter aerogenes* isolates, 29 (78.3%) were susceptible to enrofloxacin, 31 (83.8%) to sulphamethoxazole, and 28 (75.7%) to ofloxacin. All isolated *E. aerogenes* were resistant to rifampicin (Table. 12). As for *Proteus* spp, out of 12 isolates, 11 (9.1%) were susceptible to enrofloxacin, nine (7.4%) were susceptible to sulphamethoxazole and 10 (8.2%) were susceptible to ofloxacin (Table. 12). *Proteus* spp isolates were sensitive to the tested antibiotics with the exception of rifampicin in which all of the isolates showed resistance. Reported literature show that many environmental bacteria are multidrug-resistant and represent a reservoir of ancient antibiotic resistance determinants, which have been linked to genes found in pathogens (Spanogiannopoulos *et al*.; 2014).

These results indicate that the coliforms investigated in this study (*Escherichia coli, Enterobacter aerogens,* and *Proteus* spp,) were highly sensitive (>75 %) to enrofloxacin, sulphamethoxazole and ofloxacin but resistant to rifampicin. A study conducted by Igbeneghu and Abdu (2014) in Nigeria reported that *Enterobacter* spp isolated from the leaves of *Teliferia occidentalis* showed resistance to ofloxacin ranging from 8 % to 100 %.

In India about 20 % of drug resistance for *E. coli* from leafy vegetable and salads was reported (Rasheed *et al*., 2014). Gram negative bacterial isolates including *E. coli* and *Enterobacter* spp obtained from the sewage discharge of four hospitals in Saudi Arabia reported that 82.9% of the isolates were resistant to 25 µg/ml of sulphamethoxazole among the other tested drugs (Ibrahim *et al.*, 2010). About 33 % of *E. coli* isolates were resistant sulphamethoxazole. This is contrary to the *E. coli* that were isolated from the African indigenous vegetables and water sources in this study which showed no resistance to this drug.

In urban areas of Tanzania, vegetable farming is practically close to human settlements, sewage discharge, industrial effluent, rivers, streams and shallow wells (Plate 6). These observations and the results obtained in this study suggest that further studies, including molecular characterisation of the bacterial species, should be carried out for different African indigenous leafy vegetables and their related contamination sources in order to sufficiently explain the antibiotics sensitivity or resistance patterns of the bacterial isolates.

Sensitivity patterns of *S. albus* and *Bacillus* spp isolates to the selected antibiotics (enrofloxacin, sulphamethoxazole, ofloxacin and rifampicin) are presented in Table 13. Results showed that *S. albus* isolates were sensitive to enrofloxacin and resistant to ofloxacin and rifampicin. Also, most of the *Bacillus* spp were resistant to the tested drugs, with 18 (58 %) for enrofloxacin and 31 (100 %) for sulphamethoxazole, ofloxacin and rifampicin. Hellmark *et al*. (2008) reported resistance of *Staphylococcus* spp isolated from patients to sulphamethoxazole and rifampicin. According to Threfall *et al.* (2000), the resistance of these isolates could have been caused by humans through overuse or abuse of antibiotics which create a selective pressure for antibiotic-resistant organisms. In Bangladesh and India, 18 to 33 % *Bacillus* spp and 48 % *Staphylococcus* spp isolated from street food and water samples were resistant to rifampicin (Nipa *et al*., 2011).

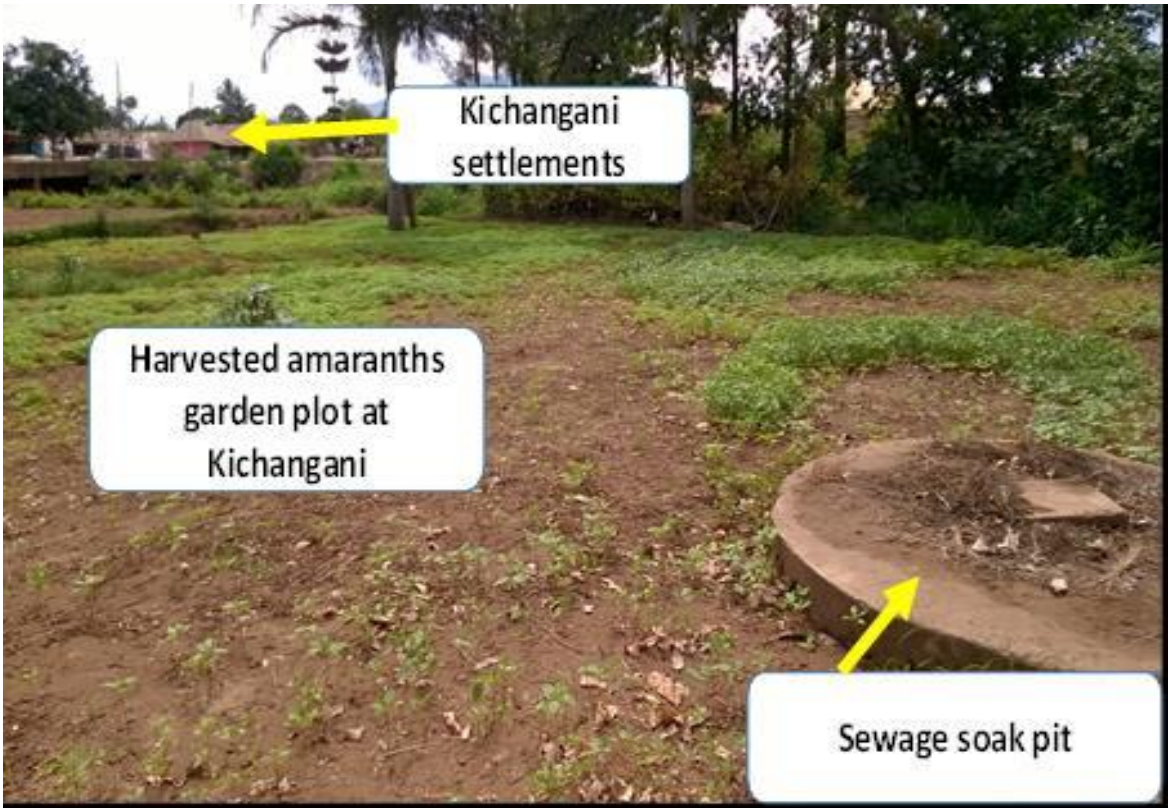


Plate 6: Amaranth vegetable garden plot at Kichangani in Morogoro Municipality neighbouring sewage soak pit and settlements

Table 13: Sensitivity patterns of *S. albus* and *Bacillus* spp isolated from different leafy vegetable types and sources using disc diffusion method

Antimicrobial agent	*Staphylococcus albus*			*Bacillus* spp		
	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)
Enrofloxacin R = ≤ 16 I = 17 – 20 S = ≥ 21	0(0.00)	0(0.00)	4(100)	13(41.9)	18(58.0)	0 (0.00)
Sulphamethoxazole R = ≤ 10 I = 11 – 15 S = ≥ 16	3(75)	1(25)	0(0.00)	0(0.00)	31(100)	0(0.00)
Ofloxacin R = ≤ 12 I = 13 – 15 S = ≥ 16	0(0.00)	4(100)	0(0.00)	0(0.00)	31(100)	0(0.00)
Rifampicin R = ≤ 16 I = 17 -19 S = ≥ 20	0(0.00)	4(100)	0(0.00)	0(0.00)	31(100)	0(0.00)

I = Intermediate, R = Resistant and S = Sensitive

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Microorganisms are abundant on the surfaces of the African indigenous leafy vegetables at different farm sites and market outlets in the study area with nightshade washings having an average (1.8×10^5 CFU/ml) while amaranth and sweet potato leaves carrying (1.7×10^5 CFU/ml) and (1.5×10^5 CFU/ml) respectively.

As for river irrigation water drawn from lower sections of the Morogoro river, bacterial contaminants (1.8×10^5 CFU/ml) were significantly ((P = 0.03) higher when compared to the middle (1.3×10^5 CFU/ml) and upper (0.8×10^5 CFU/ml) river sections. Higher total bacterial counts (1.9×10^5 CFU/ml) were also revealed in soils treated with manure at different farm sites in the study area. Isolates potentially hazardous to the health of the consumers include: *E. coli, E. aerogenes, Proteus* spp, *S. albus* and *Bacillus* spp.

This study has further provided evidence of contamination of the water used to irrigate the African indigenous leafy vegetables and the soil treated with animal manure which is used as organic fertilizer for the vegetables. These results have further demonstrated that the coliforms investigated in this study (*E. coli, E. aerogenes, Proteus* spp) were sensitive (>75 %) to the tested antibiotics (enrofloxacin, sulphamethoxazole and ofloxacin antibiotics) but resistant to rifampicin, while the non coliforms (*S. albus* and *Bacillus* spp) were intermediate resistant to the antibiotics.

5.2 Recommendations

The findings of this study have elucidated on the bacterial load found on African indigenous leafy vegetables (amaranths, nightshade and sweet potato leaves) their possible sources and their sensitivity to commonly used antibiotics as an important step towards improving food hygiene. However, this study has put in evidence a situation that necessitate interventions to reduce potential health hazard to consumers of African indigenous leafy vegetables, as briefly outlined below:

In urban areas, vegetable farming takes place in built up areas: including human settlements close to sewage discharge, industrial effluent and sources of water such as rivers and shallow wells. This practice is a threat to the health of the consumers and should be discouraged.

Education must be imparted to vendors and consumers of raw vegetables and semi prepared foods like salads of the danger of infection and hence the need to wash vegetables thoroughly with safe water, avoid eating raw/semi prepared vegetables and disinfection of the African indigenous leafy vegetables in order to protect the health of the consumers.

Microbiological control of the African indigenous leafy vegetables cultivated in Morogoro Municipality is highly recommended in order to prevent food borne diseases. This is due to the fact that most of these vegetables are consumed as salads or semi prepared snacks. The procedures used for food inspection by the Morogoro Health Department should be extended to include also leafy vegetables.

Further studies should broaden the spectrum of isolates and their characterization by molecular techniques and also to search for antibiotics to which these isolates are sensitive.

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