KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

MICROBIAL CONTAMINATION OF AN INDIGENOUS LEAFY VEGETABLE, ROSELLE (*Hibiscus sabdariffa* L.) AND ASSOCIATED RISK FACTORS ON FARM AND MARKET SAMPLES IN THE KASENA-NANKANA EAST MUNICIPALITY OF THE UPPER EAST REGION

BY:

GERARD ATAOGYE

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A THESIS SUBMITTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE (M.Sc. ENVIRONMENTAL SCIENCE)

BY

GERARD ATAOGYE

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DECLARATION

I hereby declare that this thesis document submitted to the Board of Postgraduate Studies, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana is the result of my own research work. Except for references to other people works which have been acknowledged, this has not been presented for any other degree elsewhere.

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DEDICATION

I dedicate this thesis to my children, Fabian Ataogye, Barbara Ataogye and Frederick Ataogye. It is my hope and prayer that my children grow to become academic giants.

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ABSTRACT

The microbial quality of irrigation water sources and Roselle vegetable leaves produced and sold in the Kasena-Nankana East Municipality of the Upper East Region of Ghana were evaluated by measuring the levels of total and faecal coliforms, *Enterococci* and *Escherichia coli* in the different irrigation water sources and on the Roselle leaves (fresh and dry) using standard microbiological methods. The results show that microbial numbers in the dam water used as irrigation water in the cultivation of Roselle leaves compared to the other water sources was highest in total coliforms (1.54×10^8cfu), faecal coliforms (2.87×10^6cfu), *Enterococci* (253cfu) and *E. coli* (6.85×10^5cfu). General mean microbial indicator numbers followed in a descending order in well 3, canal, well 2 and well 1 being the lowest. Generally, microbial numbers in the irrigation water sources from the canal, well 1, well 2 and well 3 were not significantly different from each other ($P>0.05$). Similarly, microbial indicator numbers on the Roselle leaves were highest on that produced with dam water followed by well 2 canal, well 3 and well1. Microbial numbers on freshly harvested Roselle leave samples were not significantly different from that of the dry leaves sold on the market. The microbial quality of the irrigation water and Roselle leaves produced and sold were higher than that of WHO and ICMSF standards. Educating the public on microbial contamination and food safety will help reduce health risk associated with the consumption of such vegetables.

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LIST OF ABBREVIATIONS

ACC	Aerobic Colony Count
ANOVA	Analysis of Variance
BPW	Buffered Peptone water
EPA	Environmental Protection Agency
FDA	Food and Drugs Administration
ICMSF	International Commission on Microbiological Specifications for Food
KNEM	Kassena-Nankana East Municipality
MPN	Most Probable Number
UPA	Urban and Peri- Urban Agriculture
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

There is an increasing demand for food in many cities in developing countries due to rising urban populations. While foods like cereals can be transported from rural areas, perishable crops like vegetables lose their market value during transportation as refrigeration is scarce. Most vegetables are therefore grown in and around cities to maintain their freshness and nutrition value. In Accra, Ghana, about 1000 farmers are involved in market-oriented urban vegetable farming and the vegetables produced are eaten by 200,000 Accra residents daily (CPWF, 2011).

However, the quality of irrigation water used is poor due to contamination from untreated wastewater resulting from poor urban sanitation. This practice though beneficial in its contributions to urban food security and livelihoods, it raises also public health concerns due to the risks posed from untreated wastewater to farmers and vegetable consumers. There is evidence that indigenous vegetables offer a significant opportunity for the poorest people to earn a living, as producers and or as traders, without requiring large capital investments (Schippers, 2000).

Roselle (*Hibiscus sabdariffa* L.) is one of the most important and common indigenous leafy vegetable grown and eaten in the Upper East Region of Ghana. It is eaten green and fresh or dried and stored for future use (McClintock and El Tahir, 2004). It is one of the important species belonging to the family Malvaceae (McCaleb, 2000).

The seeds are also used in combination with any of the traditional vegetables to prepare soup. Local drink is also prepared in the form of ‘pito’ from the seeds.

Roselle has relatively short growing periods and requires low inputs, as compared to other vegetables (Chadha *et al*., 2000). They are easy to grow, as they are well adapted to the agro-ecological zone of the Kassena Nankana East Municipality (KNEM) of the Upper East Region of Ghana. The Kassena call it 'veo' and the Nankana call it 'bito.' The potential of this leafy vegetable in playing a key role in fighting hunger and reducing malnutrition cannot be over emphasized (Watson and Eyzaguire, 2002). The young leaves and tender stems of Roselle are eaten raw in salads or cooked as greens alone or in combination with other vegetables or with meat or fish (McClintock and El Tahir, 2004).

According to Ogunlesi *et al.* (2010), indigenous leafy vegetables such as Roselle, kenaf, spinach and amaranth, serve as indispensible constituents of the human diet. Their use for the preparation of soups cuts across different cultures of West Africa, as they supply the body with minerals, vitamins, certain hormone precursors as well as proteins and energy. And their consumption in diet has been reported to protect the human body from degenerative diseases. Previous studies have also shown that traditional leafy vegetables are richer in vitamins, mineral elements and crude fibers than European vegetables (Chadha *et al.,* 2000).

Roselle (*Hibiscus sabdariffa)* is native to Tropical Africa, but is widely distributed in the Tropics and Subtropics of both hemispheres, and in many areas of the West Indies, Central America and South East Asia (Ibrahim and Hussein, 2006). Seeds of Roselle are said to have been brought to the New World by African slaves. It was grown in Brazil in the 17th Century and in Jamaica in 1707 (Morton, 1987). It is highly cultivated in the savannah zones of West Africa. Mexico, Egypt, Senegal,

Tanzania, Mali and Jamaica are also important suppliers but production is mostly used domestically (FAO, 2004).

Roselle *(Hibiscus sabdariffa)* is known by different names such as Guinea sorrel or bissap in Senegal, karkadé in North Africa, Roselle or sorrel in Asia (Morton and Roselle, 1987; Glew *et al*., 1997; Lorenzo *et al*., 2000; McClintock and El Tahir, 2004; Babalola *et al*., 2001; Nyarko *et al*., 2006; Cisse *et al*., 2009a; Cisse *et al*., 2009b).

In Senegal, traditional processing of the *H. sabdariffa* calyx has been greatly improved by the establishment of many small enterprises that use it for the production of jam, concentrates and particularly for drinks or beverages. Drinks and beverages made from the plant are very popular in Senegal and mostly consumed during the month of Ramadan (Cissé *et al*., 2009a).

1.1 PROBLEM STATEMENT

In cities and peri-urban communities, wastewater is often released untreated into the environment and this ends in streams, drains, dams, wells and water holes. Most towns and urban centres have no means of treating wastewater. The need for all year-round production of vegetables in our cities makes farmers search for irrigation water which is often contaminated wastewater. Roselle, a common leafy vegetable grown and eaten in the Upper East Region of Ghana is usually eaten green and fresh or dried and stored for future use. The young leaves and tender stems of Roselle are eaten raw in salads or cooked as greens alone or in combination with other vegetables or with meat or fish. Roselle is grown using water from dams, canals, open wells which also receive a lot of runoff from agrochemicals from farms, organic

manure from humans and Livestock and leachate from refuse and recreational activities. This means of irrigation raises public health concerns due to possible contamination of the vegetable with pathogens.

1.2 JUSTIFICATION

Many African families depend so much on indigenous vegetables for their livelihood. Rural surveys in Zambia by Ogle *et al.* (1990) reported that 52-95% of respondents used traditional leafy vegetables. Despite the great value of these traditional leafy vegetables, not much research has been carried out on them especially in the area of microbial contamination.

1.3 GENERAL OBJECTIVE

The general objective is to evaluate the microbiological quality of fresh and dried Roselle leaves and irrigation water sources for total and faecal coliforms, *Enterococci, Escherichia coli and Salmonella* in the Kassena-Nakana East Municipality of the Upper East Region, Ghana.

1.4 SPECIFICOBJECTIVES

The specific objectives of the study are to determine the presence and numbers of Total and Faecal Coliforms, *Enterococci, Escherechia coli and Salmonella* on

1. fresh Roselle leaves on the farms
2. fresh Roselle leaves in the market
3. dried Roselle leaves in the market
4. irrigation water sources (dams, canals and wells)

CHAPTER TWO

LITERATURE REVIEW

2.1 VEGETABLES

According to Taura and Habibiu (2009), vegetables are mostly annual crops belonging to the group of plants called horticultural crops which are diverse in nature. And on the bases of the edible parts, leafy vegetables are the most common, next to fruit vegetables in terms of human consumption.

Leafy vegetables are usually harvested green for human consumption. When fresh they have a high water content of about 80% (N.S.P.R.I, 1992). Like many vegetables, leafy vegetables are important source of food for humans. Vegetables possess a high content of minerals and vitamins. According to Matthew (1985), green leafy vegetables are considered as good source of vitamins, minerals such as copper and iron. Fresh and minimally processed vegetables provide most of our daily requirements for vitamins, minerals and fibre. Their role in reducing the risk of lifestyle associated illnesses such as heart disease, diabetes and cancer has resulted in a further increase in their desirability and consumption. In order to benefit significantly from these health properties, the World Health Organisation (WHO) recommends an intake of 400g, or five to nine portions, of fresh fruits and vegetables per day (Matthews, 2006).

The World Health Organisation has issued reports claiming that correct fresh produce intake alone could save 2.7 million lives a year and that 31% of heart disease cases are due to an insufficient intake of such foods (Johnston *et al.*, 2006).

As a result of the WHO recommendations (WHO, 2006a), fruit and vegetable consumption increased by at least 29% per capita in the United States between 1980 and 2000 (Matthews, 2006).

An increase in salad bars and a trend towards healthier living has resulted in a much wider consumption of fresh salad products and healthier foods, and consumer demand is forcing shops to stock fresh produce that is prepared to a ready-to-eat level and also low in or completely free of preservatives (Johnston *et al*., 2006). A concern related to this increase in fresh produce consumption is the increased exposure to potentially pathogenic bacteria as well as an increase in the total number of bacteria that are ingested, both of these increasing the chance of infection (Harris *et al*., 2003).

Competition amongst producers, as a result of an increased demand for fresh produce, has led to a wide variety and availability as well as a generally high quality. Developments are constantly being made to prolong the shelf-life of the produce by better refrigeration, packaging materials as well as modified atmosphere packaging. The minimal processing that the produce is exposed to means that the pathogens transferred to the produce in the field remain and survive any washing, processing or packaging that the produce is exposed to. These microbes may even multiply if the storage conditions are within the growth range of those that are present (Francis *et al*., 1999).

It has been shown in the literature that many Ghanaian waters that are drawn from for agricultural irrigation purposes are heavily polluted and have high pathogenic loads (Obuobie *et al*, 2006). In several cases fresh produce is irrigated using this water (Germs *et al*., 2004). Concern has arisen that there could be a carryover of pathogens from the polluted urban water to the fresh produce during irrigation and

that should the bacteria survive on this produce, the risk of infection for the consumer could be high. The increase in consumption of contaminated produce can only increase the infection rate if carryover of pathogens takes place (Suslow *et al*., 2003). While posing a threat to the health of consumers, outbreaks of associated illnesses would damage the trust of the public, thereby affecting the credibility as well as the sales of all similar produce (Johnston *et al*., 2006). Outbreaks could also result in legal battles which could potentially lead to producers losing their export licenses as well as possible rejection by the local market (Suslow *et al*., 2003). For Ghana, such outbreaks could be disastrous considering that this agricultural sector is one of great economic importance and would therefore not welcome such a setback.

Consumer awareness is a slow process and the public cannot be relied on to wash or cook fruits and vegetables sufficiently to destroy any pathogens that may be present (Bruhn, 2006). With a growing fresh produce market the food and agricultural industries are facing new challenges that require attention especially in terms of protecting the consumer against microbiological hazards (Garrett *et al*., 2003).

2.1.1 Origin and Distribution of Roselle

Roselle is native to Tropical Africa, where it is commonly cultivated, and must have been widely distributed in the Tropics and Subtropics of both hemispheres, and in many areas of the West Indies and Central America has become naturalized (Morton, 1987).

The Flemish botanist, M. de L'Obel, published his observations of the plant in 1576, and the edibility of the leaves was recorded in Java in 1687. Seeds are said to have been brought to the New World by African slaves. Roselle was grown in Brazil in the 17th Century and in Jamaica in 1707 (Morton, 1987). The plant was being

cultivated for food use in Guatemala before 1840.

In Senegal, *H. sabdariffa* was introduced in the 19th century (Kerharo and Adam, 1974) and is now grown throughout the territory; mainly in the Kaolack, Diourbel, Thies, Saint-Louis and Louga regions. In these areas, a dozen varieties are grown including Vimto, Koor, Thaiand CLT 92. Indeed, with the decline of traditional crops, especially peanut, many producers are now growing bissap to improve their income in Senegal.

2.1.2 Types of Roselle

In India the sabdariffa is called 'Roselle' and all the sabdariffa types are classified into four main groups according to the extent of pigmentation present on the stem. They are full green, green pigmented, green light red and red. The leaves in 'Roselle' are generally palmate, deeply lobed and alternately borne on the stem. The plants are normally none branching and attain a height of nearly 3 to 3.5 metres with a basal diameter of 1.0 to 2.0 cm.

Many wild forms of sabdariffa are found in Uganda which closely resembles *H. machowii* and is considered as the immediate wild progenitor of sabdariffa. The actual fibre forms have developed from edible types through a long process and may be interogressed by *H. asper* and *H. machowii*. The tall fibre yielding type was isolated and described by Webster (1914) McClintock and El Tahir (2004)in Thailand as *H. sabdariffa* var. *altissimo* from some seeds received from Gold Coast (West Africa). This variety was accidentally introduced into India as a single seed in admixture with some of other consignment of seeds from Java (Indonesia). It was first described by Khan (1930) as a new type of Roselle hemp. It is clear that the species of sabdariffa consists of two distinct types, wild and cultivated. The calyces

of the wild types are fleshy and are used for making jellys and jams and produce fibre of inferior quality. The tall types with fewer branches are cultivated for fibre purpose and they belong to *H. sabdariffa* var. *altissima*.

Table 2.1: List of some Traditional Leafy Vegetables commonly eaten in the Kassena Nankana East Municipality.

No	Common name	Local name (Kasem/Nankam)	Scientific name	family
1. Roselle	Veo	Bito	*Hibiscus sabdariffa* L.	Malvaceae
2. Kenaf	Kanzaga	Berise	*Hibiscus canabinus*	Malvaceae
3. Spider plant	Nangena	Leba	*Cleome gynanda* L.	Capparaceae
4. Bitter leaves	Siwarka	Sewaka	*Venoniaamygdalina* Del.	Compositae
5. Okra leaves	Pwori-vɔɔ	Mamvorŋ	*Abelmoschuses culentus* L.	Malvaceae

2.1.3 Uses of Roselle

2.1.3.1 Culinary uses

Roselle has many uses, of which some are still under research. Tender leaves and

stalks are eaten as salad and as a pot-herb and are used for seasoning curries. The seeds have been used as an aphrodisiac coffee substitute. The leaves and calyces also serve as a very delicious beverage which is refreshing and has a major diuretic effect (Taura and Habibu, 2009).

Roselle's fruity fresh and cranberry-tasting juice produce a variety of different foods, including health foods, sauces, jellies, iced drinks, and herb teas. It is a source of a red beverage which is said to contain citric acid and salts, serving as a diuretic, in Central America (Chadha *et al*., 2000).

2.1.3.2 Medicinal uses

According to Aziz *et al*. (2007), the medicinal uses of Roselle are numerous. In Egyptian folk medicine, it is used as an antidote for lowering blood pressure, act as an antiseptic, aphrodisiac, digestive, diuretic, purgative, sedative, and tonic. It is also a folk remedy for abscesses, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments, hypertension, neurosis, and scurvy. Researchers now are doing studies to see if Roselle is indeed active in lowering blood cholesterol levels.

2.1.3.3 Industrial uses

Roselle is also cultivated for the baste fibre obtained from the stems, which has many uses including weaving jute sacks, ropes, handbags and door mats (FAO, 2007).

2.1.3.4 Production practices

Production practices, growth conditions and the location of the edible part during growth will in combination with intrinsic, extrinsic, harvesting and processing factors affect their microbial status at the time of consumption. There is an increased demand on the market for leafy vegetables. Production practices for this category of produce may involve the use of organic fertilizers and the use of alternative measures

to chemical plant protection products for the control of pests, mites and fungi. Therefore, the potential risks for contamination of leafy vegetables by faecal pathogens or by mycotoxin producing moulds have to be acknowledged (Westphal *et al*., 1987).

2.2 MICROBIAL CONTAMINATION

Microbiological contamination refers to the presence of one or more various bacteria, yeasts, mould, fungi, protozoa or their toxins and by-products, which could adversely affect the product or a consumer's health and safety (Levitt, 2000).

Leafy vegetables inflict economic loss by causing or spreading of human disease after consumption and these affect the health and the progress of the nation. Their soft textured nature makes them highly attractive to microbial invasion, and they are very susceptible to physical and microbial spoilage. The common sources of microorganisms that contaminate leafy vegetables include air, soil, farm pests, handlers and irrigation containers used (Taura and Habibu, 2009). Leafy vegetables can become contaminated whilst growing in the field or during harvest, handling, processing, distribution and use (Beuchat, 1998). Any microbial contamination present is likely to reflect the environment through which the product is obtained.

Irrigated vegetables are found to be highly contaminated with microbes that are harmful to both plants and animals including man (Matthew, 1985). Consumption of contaminated vegetables could pave the way for ingestion of considerable number of human pathogenic bacteria. This eventually could result in establishment and manifestation of diseases on humans (Francis *et al*., 1999; Taura and Habibu, 2009).

2.2.1 Pathogens Associated With Leafy Vegetables

Vegetables have been associated with outbreaks of food borne diseases in many countries. Organisms involved include bacteria, viruses and parasites (De Roever,

1998). However, they represent only a small proportion of the total number of cases reported. In an overview by ICMSF (1998) they reported that animal wastes used as fertilizer in vegetable production may contribute to as much as 20% of infections by *Shigella*, *Salmonella*, *Vibrio cholerae* and amoebas. However, there is no evidence that these countries have a particular problem with fresh produce. The number of outbreaks reported is more likely to reflect the comprehensiveness of the surveillance and reporting system operating in these countries than the incidence of disease associated with fresh produce.

2.2.2 Microbial Flora on Leafy Vegetables

Microorganisms form part of the epiphytic flora of vegetables and many will be present at the time of consumption. The majority of bacteria found on the surface of plants is usually Gram-negative and belong either to the *Pseudomonas* group or to the *Enterobacteriaceae* (Lund, 1992). Many of these organisms are normally non-pathogenic for humans. The numbers of bacteria present will vary depending on seasonal and climatic variation and may range from 104 to 108 per gram. The inner tissues of vegetables are usually regarded as sterile (Lund, 1992). However, bacteria can be present in low numbers as a result of the uptake of water through certain irrigation or washing procedures. If these waters are contaminated with human pathogens, these may also be introduced.

About two thirds of the spoilage of fruits and vegetables is caused by moulds (ICMSF, 1998).Members of the genera *Penicillium*, *Aspergillus*, *Sclerotinia*, *Botrytis* and *Rhizopus*are commonly involved in this process. The survival or growth of contaminating microorganisms is affected by intrinsic, extrinsic and processing factors. Factors of importance are nutrient composition, pH, presence of scales and fibres, redox potential, temperature and gaseous atmosphere. Mechanical shredding,

cutting and slicing of the produce open the plant surfaces to microbial attack. In many cities of SSA as in other developing regions, farming activities are found almost everywhere: behind houses, along roadsides, on roofs, along and between railway lines, in parks, along rivers, under power lines, and in high, medium and low density areas. At least 20 million West Africans currently live in urban households with some kind of urban agriculture (Drechsel *et al*., 2006).

In many cases, this production is for subsistence needs to reduce household expenses while contributing to the daily diet. Subsistence production appears to expand during economic crises and helps many poor households who spend from 60% to 80% of their limited income on food (Smith *et al.,* 1996). The United Nations Development Program estimated in 1996 that 800 million people are engaged in urban agriculture worldwide. Of these, 200 million are considered to be market producers employing 150 million people on full-time basis (Smith *et al.*, 1996).

Market-oriented production is usually informal and takes place on open urban spaces, preferably in inland valleys and lowlands with water access or close to streams and drains, which allow dry season production of highly valuable crops with corresponding profits. Also peri-urban areas often attract highly specialized irrigated systems even for foreign export taking advantage of the proximity of city airports and harbours. Examples are pineapple farmers around Accra in Ghana or Basil leaf farmers on the beaches of Lomé in Togo. Also irrigated ornamental and flower production is a common and profitable UPA system although high investment costs are needed (Drechsel *et al.,* 2006).

Depending on cultural specifics and production system these activities can have a very specific gender involvement with women in charge of production and/or marketing and often it is the only source of family income. A survey in 13 countries

of West Africa showed that in 16 of 20 cities, men are mostly involved in open-space urban vegetable farming while in most cases; women dominated the vegetable retail sector (Drechsel *et al*., 2006).

Open space urban agricultural production can become a profitable venture if market proximity is combined with water availability for irrigation. This permits dry season production and supports intensive year round production. Different sources of water are used for urban and peri-urban Agriculture in Sub Saharan Africa. In Lagos, for example, peri-urban farming depend solely on the Fadama wetland where farmers are able to cultivate continuously throughout the year using water from flowing rivers, ponds, dug wells or wash bores.

2.3 IRRIGATED URBAN AND PERI- URBAN AGRICULTURE (UPA)

Irrigated urban and peri-urban vegetable production appears as one of the most productive and income generating farming systems in Africa despite often marginal soils, insecure tenure and its informal character. The success, which is steered by the large urban market and demand for high value crops, also require high inputs in the form of water, nutrients and pesticides. While pesticide and fertilizer/manure can be bought, it is difficult to find sites with proper, reliable and cheap water access. In this situation, farmers often make use of typical urban 'resources' like water from streams or drains, exposing urban farming to urban pollution. Most farmers are not aware of their personal risk involved with the use of polluted irrigation water, or other health threats of higher priority like malaria. And in many cases, wastewater is the only reliable water source throughout the year, i.e. the basis of their livelihoods and no issue for discussion (Keraita *et al*., 2002).

Due to low industrialization, the contamination is seldom through heavy metals but

through faecal matter. Studies from Ghana, Senegal and Kenya confirmed that the bacteriological contamination of urban water sources generally exceeds irrigation standards, and can contribute significantly to crop contamination (Niang *et al*., 2002). Other problems can be soil and groundwater pollution or salinization. Thus, despite all its benefits in terms of food supply, nutrition, employment, and poverty alleviation, urban vegetable production poses human health and environmental risks which makes it struggle for official recognition, not to mention support, especially in Sub-Saharan Africa with its complex urban sanitation problems (Obuobie *et al*., 2006).

The tendency of many local governments now is to formulate more diversified and regulatory policies that seek to actively manage the health and other risks through an integrated package of measures, with the involvement of the direct stakeholders in the analysis of problems and development of workable solutions. In March 2002, the Dakar declaration was signed by seven mayors and city councilors from West Africa in support of the development of the urban agriculture sector, well recognizing the potential problems of wastewater use (Niang *et al*., 2002). Also a recent declaration on 29 August, 2003 in Harare by five Ministers of Local Government from East and Southern Africa called for the promotion of a shared vision of UPA.

However, recognition is not yet action. To support the important role of irrigated urban and peri-urban agriculture, city authorities will have to work with their farmers to find the right balance between health risk mitigation and livelihood security. There are many options also in situations where better municipal water treatment is not possible in the near future thus no possibility to meet the common irrigation water quality guidelines (Drechsel *et al*., 2006). Instead of banning urban farmers, authorities could for example allocate areas with safer water sources for farming as

done in Cotonou.

2.4 IRRIGATION METHODS AS A CARRY-OVER MECHANISM

The microbial quality of irrigation water is of importance as poor quality water can lead to the introduction of pathogens onto produce during pre and postharvest activities. Because of this problem, indirect or direct contamination of produce from water of persistent pathogens on harvested vegetables has been long recognized as a potential hazard (WHO, 2005). Though reports on direct evidence of food-borne illness due to contamination of fresh produce during "commercial" production are more limited, many of these crops have been implicated in food borne illnesses.

Garcia *et al.* (1987) showed that under commercial conditions, 181 irrigation samples and 859 vegetables irrigated with the same water source in Spain were found to be contaminated with *Salmonella typhimurium; S. kapemba; S. london*and *S. blockey* serotypes.

Different irrigation methods have been found to correlate with the level of microorganisms present on produce (FDA, 1998). It has also been reported that the transfer of microorganisms from irrigation water to produce is dependent on the nature of the produce (Beuchat and Ryu, 1997). Spray irrigation could be expected to increase the risk of contamination in comparison to drip irrigation or flooding because leafy vegetables provide large contact surfaces for water and for the attachment of microorganism (Sadovski *et al*., 1978).

Other popular methods are drip irrigation, subsurface drip irrigation or furrow irrigation; all of which result in minimal splashing of water and thus minimal exposure of the edible produce to potentially-contaminated water (Johnston *et al*.,2006). These methods could then be used in cases where contamination of

produce is a real threat and contact between the water and the produce is preferred to be kept to a minimum. Irrigation choices should also take factors such as water quantity, cost, soil type, slope of the field and the type of crop rotation system into account. These factors must be weighed up against the likelihood of pathogen contamination and a decision must be made for each specific situation (Mena, 2006).

Different researchers have evaluated the presence or persistence of pathogens conveyed to crops by spray irrigation, irrigation by sewage effluent or drip irrigation (Sadovski *et al.,* 1978; Garcia *et al.,* 1987). It was found that carry-over varied and was depended upon the level and nature of environmental stress. Carry-over was correlated to target population densities in the source water and spatial orientation relative to the point source. The level of organic matter in the water also impacted the survival of pathogens.

Irrigation water polluted with manure has also been implicated in the outbreaks of enterohaemorrhagic *E. coli* 0157:H7 infections (Kim *et al*., 2006). The infections were associated with lettuce and other leaf crops and they are occurring with increasing frequency (Mahmoud *et al*., 2007). However, it has been found that *Salmonella* became undetectable on effluent-irrigated lettuce five days after irrigation was terminated, but *E. coli* indicator strains persisted (Mukherjee *et al.*, 2004). It was reported by Matthews, (2006) that in the USA spray/overhead irrigation resulted in a greater number of lettuce plants‟ testing positive for *E. coli* O157:H7 at harvest following a single exposure to the pathogen. Similarly in Nigeria lettuce and carrots were positive for *Salmonella*, *Vibrio* spp. and *E. coli* following irrigation with water that tested positive with the same pathogens (Matthews, 2006). Mahmoud *et al.* (2007) reported that strawberries tested positive for the presence of *E. coli* after irrigation by both drip and overhead methods were use.

Contaminated irrigation and surface run-off waters and the use of sewage as a fertilizer can also be sources of pathogenic microbes that contaminate fruits and vegetables in the field (Beuchat and Ryu, 1997). It was also found that with sewage contamination between 84 and 100% samples were contaminated with either *L. monocytogenes*or *L. innocua* during a two year sampling period. *Salmonella* was also present in more than 50% of irrigation water samples contaminated with raw sewage or primary treated chlorinated effluents (Wang *et al.*, 1996).

It has been found that cholera and typhoid microbes can also be transferred during the irrigation of vegetables with untreated wastewater. Therefore, in areas where rivers are known to test positive for such pathogens, the method of irrigation as well as the option of water treatment should be critically considered.

Farming conditions and practices play a critical role in the contamination of produce and it is usual for the level of contamination to have dropped substantially from when it is harvested to the time of consumption (Francis *et al*., 1999). Temperature is one of the most important factors that influence the growth, survival or decay of bacteria on produce after harvest. Each group of bacteria has its own growth criteria and therefore different bacteria will react differently under the presiding conditions (Peleg, 2000). In contrast to the usual decay patterns, *Listeria monocytogenes*as well as non-proteolytic strains of *Cl. botulinum*and *Aeromonas*are psychrotrophic and *Aeromonas* have been found to increase by 1 log value after 7 days at 3-4°C (Francis *et al*., 1999). This means that there is still a chance for some of the contamination pathogens to multiply on produce after harvest.

2.5 WATER QUALITY

When assessing the safety of produce the term, water quality is based on the pathogenic load of the water as a measure of quality (WHO, 1989). This term is more generally used when determining the efficacy of a treatment process on a water sample. In the case of produce safety it is the pathogenic load that is determined, rather than measuring chemical parameters (Carr, 2005). Water quality as described above is important as it dictates for what purposes the water is suitable (WHO, 1989). There are five different categories into which pathogens are classified according to their survival characteristics. Categories 1, 3, 4 and 5 include the nematodes, helminths, protozoa and viruses while Category 2 contains the bacteria. These bacteria are consider those that are infective immediately upon excretion but can still multiply outside of the host and generally have a higher median infective dose than the other four pathogen categories (Carr, 2005). More recently thermo tolerant *E. coli* evaluation has become one of the major tools used world-wide to determine the microbial quality of water (WHO, 2006b).

The high usage of fresh water could create problems if the water is heavily contaminated with microbes especially where there are no alternative water sources available. It is therefore of utmost importance that the microbial quality of Ghana's fresh water resources be maintained.

2.6 WATER STANDARDS

In order to be able to ensure that water will be sufficiently safe for its intended use, it has been necessary to construct a set of guidelines for a variety of uses and all water

should comply with the regulations and guidelines pertaining to its intended use. In terms of the microbiological quality of water, guidelines for faecal coliforms are given, depending on the method used, as the maximum permissible number of colony forming units per 100 mL water (cfu.100 mL^{-1}) (WHO, 1989). Both the World Health Organisation (WHO) and the Environmental Protection Agency (EPA) have guidelines for the quality of irrigation water. They both recommend that water used for the irrigation of fresh produce should have a faecal coliform load of less than 1000 cfu per 100 mL^{-1} (WHO, 1989). This applies to all water being used for the irrigation of crops, irrespective of its source.

The current guidelines for *E. coli* in irrigation water are not more than 1000 organisms per 100 mL^{-1} (WHO, 1989; WHO, 2006b). From the literature there is no clear indication as to how the value of <1000 *E. coli* per 100 mL was reached but it is considered as a very conservative maximum. However, it is yet to be tested at what point and in what quantity carryover of pathogens from irrigation water to fresh produce takes place. It is interesting to note that the permissible load of *E. coli* on raw fruits and vegetables is zero per g product. Therefore if *E. coli* present in irrigation water is carried over onto produce, the produce should be considered as suspect.

There are no many published limits or guidelines available for the total number of microorganisms and where limits do exist, the values vary greatly. The total number of microorganisms is determined by performing an aerobic colony count (ACC) on a water sample. Since the organisms detected are not necessarily harmful to the produce or to the consumer, the value obtained from this test is used to indicate number of possible pathogens or spoilage bacteria. Further tests should be performed if more specific information is required regarding the different microorganisms

present. However, if the ACCs are high, there is a greater chance that there are corresponding high levels of spoilage organisms or pathogens. Similarly, a low total load usually reflects very low levels of spoilage organisms or pathogens, if there are any. The result for the ACC can, therefore, in broad terms serve as an indication of contamination. In literature, recommended limits for ACCs range from 2.9 to 7.3 log cfu.g-1. When assessing the safety of food, the lag time, exponential growth phase and the decay/survival rates of the organisms present on the products are important.

In terms of food spoilage, a product is considered to be spoiled when its microbial load exceeds 1 x 10^{5-7} organisms per gram (Geldreich, 1996). This limit does not make an allowance for the presence of pathogens. The infective doses of pathogens can be as low as 1-10^{3} organisms, so a product can appear to be unspoiled while it is actually carrying dangerous levels of pathogens. The infectivity of the *Escherichia coli* pathogenic strains is substantially higher than that of the other strains. As few as 100 EHEC organisms can cause infection (WHO, 2006b). For this reason, it is important to test the quality of irrigation water and fresh produce regularly; although in practice it is not always possible (WHO, 2006b). Clearly, the microbial contaminated water can pose a big threat to food safety if produce is unknowingly being infected with pathogens while being sold. For this reason, WHO has drawn up a set of standards that dictate the legal maximum loads for different pathogens that can be present on a product (WHO, 2006b). While national and international standards and regulations are published and enforced by, or should be, official authorities, there are also certain standards that are set by the industry itself.

The benefit of such industry standards is that they are usually manageable and deal with food safety problems faced in the specific industry through the sharing of information. The role of inspections and certifications by third party groups is a

further means of insurance and assurance for retail food companies. It also provides a guarantee of a certain level of quality to the client and prevents each client from independently needing to inspect the supplier prior to purchase (Michaels and Todd, 2006).

2.7 SOURCES OF CONTAMINATION

The two main categories of contaminants are chemical and microbiological with the latter being the focus of this particular study. For this reason, further discussions will only include aspects of microbiological contamination. However, chemical contamination is equally important and the health risks, both short-term and chronic are serious and not to be neglected. The origin of chemical contamination and methods of detection differ greatly from microbiological contaminants and are thus generally studied separately.

Most of the microbiological contaminants posing a threat to the health of consumers originate from humans or animals, with the majority of these being of faecal origin or transferred through faeces (Harris *et al*., 2003). According to Jamieson *et al.* (2004) and Maciorowski *et al.* (2007) contamination of water can be divided into two major mechanisms namely point-source and non-point source contamination. Point-source contamination emanates from a clearly identifiable point such as animal feedlots as well as from runoff from storage facilities. Non-point-source contamination can be from different sources or even many points. For example this type of contamination can occur, at the site of manure application, at a surface level, at the site where the manure is actively combined with the soil, and at the site where the origin of the manure is from livestock (Jamieson *et al.*, 2004; Maciorowski *et al.*, 2007).

The use of manure as a fertilizer has increased in popularity as consumers are seeking fresh produce that has been produced without harmful or chemically loaded pesticides and fertilizers (Suslow *et al*., 2003). The alternative to fertilizer is manure and its use in the field is increasing mainly as a result of consumers "organic" trends to enhance health. Ironically' it is only for conventional fertilizers that the microbiological safety can be assured and the use of fertilizer is, in fact, much less risky than manure. Manure is likely to be loaded with bacteria present in animal faeces and the risk of contamination of irrigation water is high. Thus a potential hazard does exist for the carry-over of potential pathogens to fresh produce directly or indirectly through contaminated irrigation water.

Another pathway for faecal contamination is through direct contact with human sewage. Bio-solids or sewage sludge are what remains after the liquid phase of sewage has been removed for treatment. This has been used as a fertilizer or added to nutritious slurries for crops (Minhas *et al*., 2006). However, this has been recognized to be potentially heavily loaded with pathogens and has been outlawed by the British Retail Consortium (Coetzer, 2006). While animal manure is a problematic contaminant that is very difficult to control, contamination of rivers and water systems with human faeces is an enormous problem that is on the increase. Human faeces is entering river systems through failing sewage pipes and treatment plants, illegal release of untreated sewage and the close proximity of informal settlements with no sanitation facilities to river resources which become the obvious dumping ground for the generated waste (Barnes and Taylor, 2004). Until these informal settlements are provided with functioning sanitation facilities and are trained to use them properly, little change for the better can be expected and these communities have no option but to continue dumping their waste into gutters and nearby water

bodies.

While informal settlements are responsible for some faecal pollution and even waste dumping, they are, by far not the only guilty party. For example, it was reported by Barnes, (2003) that a winery downstream of the Kayamandi settlement was dumping cellar and production effluent into the rivers. Depending on the fermentable carbon load of this type of pollution it might result in increased fermentation in the rivers. The acidity and conditions of the water would therefore also be changed, thereby allowing organisms which would not normally be able to survive in river water to grow and multiply.

When considering the increasing pollution of Ghana water bodies and the downward trend in water quality, it is clear that the situation will hardly improve unless a control body or regulatory agency takes charge and enforces the quality standards of water bodies from which are drawn from for irrigation.

While fruits and vegetables are in the field, polluted water is one of the major threats for product contamination. The produce can be exposed to water during both irrigation and application of pesticides, and the water used for these purposes can be drawn from gutters, streams, open ditches or canals, dams or ponds, or reservoirs. Alternatively, if available, municipal water can be used but the quality of this water cannot always be relied on (Johnston *et al*., 2006).

Another source of contamination is the land on which the produce is grown. In some cases, farms have been acquired without knowledge of its previous purpose and if it was used for animals, or was even loaded heavily with manure, then the reservoir that has built up in the soil can potentially contaminate the produce (Coetzer, 2006). In the case of farms positioned near rivers, the land use upstream is also important

for the safety of the plot. For example, during times of flooding, contaminants that are carried by the river from various sources upstream can be washed onto land that the river does not usually reach and result in unexpected and irreversible contamination.

Contamination of fresh produce can also take place postharvest (Harris *et al*., 2003). In a food safety review, Harris *et al.* (2003) reported that numerous microbial pathogens have been isolated from fresh fruits and vegetables but not all were linked to produce associated illnesses. Many of the isolated organisms have the potential to under the right conditions cause illnesses. Vehicles of postharvest microbial transmission include harvesting equipment, packing house conditions, unhygienic workers, processing plants and even pests (in the field or postharvest) (Matthews, 2006; WHO, 2006b). In the packing house, transmission of pathogens through practices such as washing can occur if the water is not properly disinfected, filtered or replaced on a regular basis. It is thus important that, producers acknowledge the role that the origin and pollution level of the irrigation water can play in the safety of the end product.

2.7.1 Coliforms

Coliforms are defined as being Gram-negative, non-spore forming, rod-shaped facultative anaerobes that are part of the family *Enterobacteriaceae* (Leclerc *et al*., 2001). Coliforms are also characterized by their ability to ferment lactose at 35°C, resulting in gas formation. Approximately 10% of all intestinal microorganisms including *E. coli* fall into the coliform group. However, this group is not exclusive to intestinal bacteria and it has thus been broken down into smaller sub-groups in order for the intestinal bacteria to be able to be classified separately.

2.7.1.1 Total coliforms

There are several other genera not part of the coliforms that can ferment lactose and possess beta-galactosidase and can yield false total coliform reactions. A major limitation of using the coliforms as indicator is the classification which presents major problems as a result of the high degree of character variation extending from the lactose positive/negative variations to the highly reactive *Enterobacter*genus.

2.7.1.2 Faecal (thermotolerant) coliforms

The faecal coliforms are considered a sub-group of the total coliforms. Many of them are mesophiles and capable of growing and producing acid from lactose at 44.5°C. These are generally considered to be the thermotolerant. This temperature tolerance is specific to those coliforms many of which are adapted to survive within the intestine of a warm-blooded host. Beside *E. coli,* several species of the genera *Klebsiella, Enterobacter, Citrobacter, Hafnia, Pantoea, Raoultella*and *Serratia*also fall into the faecal coliform group and many are thermotolerant (Leclerc *et al*., 2001). However, members of these genera are also present in the environment and their presence in water and produce is not necessarily related to faecal contamination (Alonso *et al*., 1999). Thus, the specificity of faecal coliforms as indicators of faecal pollution varies considerably depending on environmental conditions. While the presence of faecal coliforms is often indicative of faecal pollution, more specific tests have been developed to detect which coliforms are present.

2.7.2 Escherichia coli

The *Escherichia coli* group is one of the most common indicator organisms and is used particularly for the detection of faecal contamination, especially in drinking

water. The presence of *E. coli* is never beneficial to a consumer and always points to the possibility of faecal contamination. Its presence, therefore, should not be ignored if it is detected in a sample.

Escherichia coli is of the family *Enterobacteriaceae* and most strains are normal inhabitants of the intestinal tract and are practically always present in faeces and thus also in faecally contaminated water. This has resulted in the almost universal use of *E. coli* as the standard indicator for faecal contamination (Francis *et al*., 1999). There are also several reports in the literature confirming the presence of *E.coli*and other thermotolerant coliform bacteria in the environment. Not all strains are harmless and major pathogenic strains like *E. coli* O157:H7, have been identified in several MPF-related food outbreaks. According to Francis *et al*. (1999), if ingested, this strain can result in haemorrhagic colitis, gastroenteritis and kidney failure, while it less commonly results in thrombocytopenic purpura and haemolytic uremic syndrome (Gil and Selma, 2006). Serious cases can even result in death. The monitoring of faecal matter in rivers and on the MPFs is therefore of great importance since there is very little control possible over animal faeces entering the river (Francis *et al*., 1999).

E.coli has been reported to be the most sensitive thermotolerant coliform to environmental stresses and does not usually grow outside the human or animal gut. In contrast, it has also been reported that the general survival ability of *E. coli* increases upon exposure to one environmental stress which indicates that it is able to activate survival mechanisms when it is threatened (Maciorowski *et al*., 2007). *Escherichia coli* are known to be able to withstand very highly acidic environments and can survive at pH ranges as low as 3.3 - 4.2. The number of *E. coli* present in an environment was found to increase logarithmically with an increase in oxygen,

indicating that *E. coli* requires high levels of oxygen for metabolism and therefore grows better under conditions of high atmosphere (Maciorowski *et al*., 2007).

Unfortunately, the methods used to determine the presence of *E. coli* for use as indicator of faecal contamination also have their limitations. There are several other coliform species that can ferment lactose even at 44.5°C, grow on McConkey agar, are glucuronidase-positive and are indole-positive making a positive identification of *E. coli* difficult.

2.7.3 Enterococci

Enterococci are facultative anaerobic organisms, that is, they are capable of cellular respiration in both oxygen-rich and oxygen-poor environments. Though they are not capable of forming spores, enterococci are tolerant of a wide range of environmental conditions: extreme temperature (10-45°C), pH (4.5-10.0) and high sodium chloride concentrations. *Enterococci t*ypically exhibit gamma-hemolysis on sheep's blood agar. *Enterococcus* is a genus of lactic acid bacteria of the phylum Firmicutes. Enterococci are Gram-positivecocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from streptococci on physical characteristics alone. Two species are common commensal organisms in the intestines of humans: *E. faecalis* (90-95%) and *E. faecium* (5-10%). Rare clusters of infections occur with other species, including *E. casseliflavus*, *E. gallinarum*, and *E. raffinosus* (Gilmore MS, et al, ed. 2002).

Members of the genus *Enterococcus* were classified as Group D *Streptococcus* until 1984, when genomic DNA analysis indicated a separate genus classification would be appropriate (Schleifer KH; Kilpper-Balz R 1984). Important clinical infections caused by *Enterococcus* include urinary tract infections, bacteremia, bacterial

endocarditis, diverticulitis, and meningitis (Fisher K, Phillips C, 2009). Sensitive strains of these bacteria can be treated with ampicillin and vancomycin (Pelletier LL Jr. 1996). From a medical standpoint, an important feature of this genus is the high level *of* intrinsic antibiotic resistance (.Ryan KJ, Ray CG, ed. 2004).

2.7.4 Salmonella

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella which grade in all directions (i.e. peritrichous). They are chemo-organotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes. Most species produce hydrogen sulfide, which can readily be detected by growing them on media containing ferrous sulfate, such as TSI. Most isolates exist in two phases: a motile phase I and a non-motile phase II. Cultures that are non-motile upon primary culture may be switched to the motile phase using a cragie tube (Clark MA, Barret EL, 1987).

Salmonella is closely related to the *Escherichia* genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses like typhoid fever, paratyphoid fever, and food-borne illness (Ryan KJ, Ray CG 2004).

Salmonella infections are zoonotic and can be transferred between humans and non-human animals. Many infections are due to ingestion of contaminated food. A distinction is made between enteritis Salmonella *and* Salmonella thyphoid/paratyphoid *Salmonella*, where the latter - because of a special virulence factor and a capsule protein (virulence antigen) - can cause serious illness, such as *Salmonella enterica subsp. enterica*sero var Typhi. *Salmonella typhi* is adapted to

humans and does not occur in other animals.(Jantsch *et al.,* 2011). *Salmonella* species are facultative intracellular pathogens that enter cells via macropinosomes (Kerr et al., 2010).

2.7.5 Enteritis *Salmonellosis* or Food Poisoning *Salmonella*

This is a group consisting of potentially every other serotypes (over a thousand) of the *Salmonella* bacterium, most of which have never been found in humans. These are encountered in various *Salmonella* species, most having never been linked to a specific host, and can also infect humans. It is therefore a zoonotic disease. The organism enters through the digestive tract and must be ingested in large numbers to cause disease in healthy adults. Gastric acidity is responsible for the destruction of the majority of ingested bacteria. The infection usually occurs as a result of massive ingestion of foods in which the bacteria are highly concentrated similarly to a culture medium. However, infants and young children are much more susceptible to infection, easily achieved by ingesting a small number of bacteria. It has been shown that, in infants, the contamination could be through inhalation of bacteria-laden dust. After a short incubation period of a few hours to one day, the germ multiplies in the intestinal lumen causing an intestinal inflammation with diarrhea that is often muco-purulent and bloody. In infants, dehydration can cause a state of severe toxicosis. The symptoms are usually mild. There is normally no sepsis, but it can occur exceptionally as a complication in weakened elderly patients (Hodgkin's disease, eg.). Extra-intestinal localizations are possible, especially *Salmonella* meningitis in children, osteitis, etc. Enteritis, *Salmonella* (e.g., *Salmonella enterica* subsp. *enterica*sero var *enteritidis*) can cause diarrhea, which usually does not require antibiotic treatment. However, in people at risk such as infants, small children, the elderly, *Salmonella* infections can become very serious, leading to complications. If

these are not treated, HIV patients and those with suppressed immunity can become seriously ill. Children with sickle cell anaemia who are infected with *Salmonella* may develop osteomyelitis (Jantsch et al.,2011).

In Germany, *Salmonella* infections must be reported (§ 6 and § 7 of the German law on infectious disease prevention, *Infektionsschutzgesetz).*

Between 1990 and 2005, the number of officially recorded cases decreased from approximately 200,000 cases to approximately 50,000. It is estimated that every fifth person in Germany is a carrier of *Salmonella* (Cummings et al., 2010). In the USA, there are approximately 40,000 cases of *Salmonella* infection reported each year. According to the World Health Organization, over 16 million people worldwide are infected with typhoid fever each year, with 500,000 to 600,000 fatal cases.

Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment (Clark MA, Barret EL, 1987) However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness (María et al., 2011 *).*

CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLING SITES

The research was conducted in the Kasena-Nankana East Municipality and its suburbs. The district covers a land area of 1,674 square kilometres with a population of about 149,680 (Ghana Statistical Service, 2010).Three farm sites which uses different water sources (canals, dams and wells) for irrigation and the main market (Navrongo market) in the municipality were selected for sampling. The three farm sites were Bonia, Doba and Pongu.

3.2 SAMPLING

Fresh Roselle leaves and water samples obtained from the three farm sites in Bonia (canal irrigation), Doba (dam irrigation) and Pongu (wells irrigation) and fresh and dry leaves samples from the Navrongo market were used in the study. Monthly samples were collected from each farm, irrigation water source and market sites from January to April 2012.

3.2.1 Roselle Leaves Sampling

On each sampling date, five cultivated beds were randomly selected at each of the three farm sites and fresh plants of Roselle leaves were taken across the diagonals using a sterile scissors into sterile polythene bags and transported in an ice chest to Kwame Nkrumah University of Science and Technology Microbiology Laboratory where they were analyzed within 24 hours.

Plate 3.1: Roselle irrigated fields

Plate 3.2: Roselle ready for harvesting

Plate 3.2: Roselle ready for harvesting

3.2.2 Market Sampling

Fresh Roselle leaves were also collected from each of the sellers that were selected at the market. At the market, samples were collected under normal purchase conditions from five randomly selected sellers at midday to ensure that the vegetables had received enough splashing with the refreshing water. Five Roselle plant leaves were collected from the upper, middle and lower basins from each of the five sellers given a total of twenty five Roselle plant leaves per market day. The samples were taken four times at four different market days. The dry samples were also collected from five sellers randomly selected by picking from the top, middle and the lower parts of the selling receptacle.

Plate 3.3: Washing of Roselle in irrigation water

Plate 3.4: Harvested Roselle ready for the market

Plate 3.5: Fresh Roselle leaves sold at the market

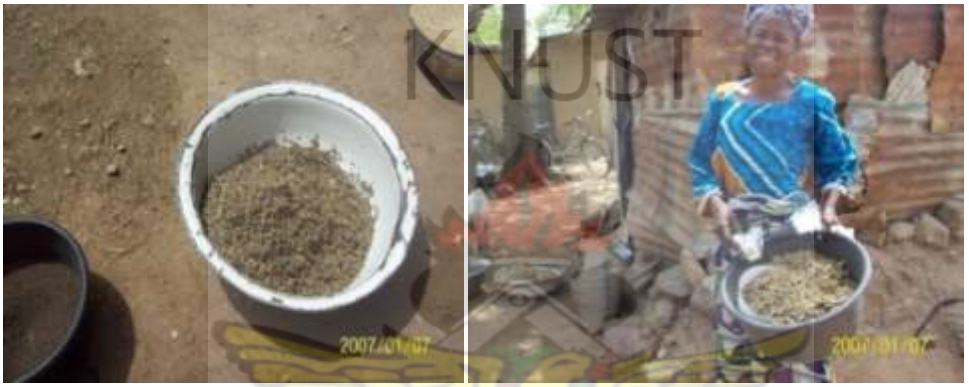

Plate 3.6: Dry Roselle leaves sold at the market

3.2.3 Irrigation Water Sampling

Water samples were collected from each irrigation water source (canal, dam and wells) from the three farm sites in the Kassena-Nankana East Municipality. Sampling at all sites was carried out four times between eight and nine in the morning in keeping with farmer's irrigation practices. At the canal, sterile 500-ml bottles were used to take water samples from three different points at 20-m intervals along the canal. At each interval, the water body was disturbed just like the farmers do before the bottle is immersed, opened and the bottle filled and closed before lifting the bottle out of the water. The dam water was also collected at different intervals by disturbing the water body first and then immersed the bottle in the water

before the lead was opened and then closed when the bottle was filled before taking it out from the water. The well water was taken from the containers which were used by the farmers just before they pour the water on the Roselle vegetables. The well water was taken from the three most used wells. Samples from the three sites were later transported to the laboratory in an ice chest and analyzed within 24 hours.

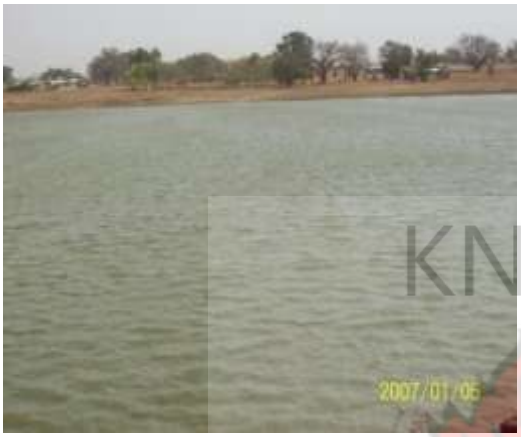

Plate 3.7: Dam water source

Plate 3.8: Canal water

Plate 3.9: Well 1 water source

Plate 3.10: Well 2 water

Plate 3.11: Well 3 water source

3.3 MICROBIOLOGICAL ANALYSIS

Total and Faecal coliforms were estimated using a three-tube Most Probable Number (MPN) method according to standard procedures (Anon, 1992). Twenty (20g) grams of the Roselle leaves was placed in a stomacher bag and pulsified in 180 ml of distilled water for about 15 seconds using a pulsifier (PUL 100E; Stuart Scientific Co. Ltd, U.K). One milliliter of the stomacher bag content was used preparing serial dilutions (10^{-1} to 10^{-7}). One milliliter of each dilution was inoculated in triplicate into 5 ml of MacConkey broth and incubated for 24 hours at 37^{o}C and 44^{o}C for Total and Faecal coliforms, respectively. There was a colour change in some of the Tubes from purple to yellow which indicated the presence of the coliforms. All positive tubes were recorded as positive for Total and Faecal coliforms and estimated counts were obtained from MPN tables (Collins *et al.,* 1989) and results expressed in per gram wet weight for Roselle fresh leaves and 100 ml for irrigation water and then the dry samples results were expressed in per gram dry weight.

Enterococci were enumerated by placing 1 ml volumes of the raw content from the bag and 1ml volume of the serial dilutions (10^{-1}) prepared for the thermotolerant

coliforms directly onto two separate set plates of Slanetz and Bartley agar. These were allowed to dry and then incubated for 4 hr at 37°C and for 44 hr at 44°C. Red, maroon or pink colonies were then counted using the colony counter. All counts were expressed as colony forming units (cfu) per gram wet weight for Roselle leaves and per 100 ml for water.

Salmonella was enumerated by the MPN technique (Anon, 1992) using Buffered Peptone water (BPW) as a pre-enrichment medium (Moringo *et al*., 1989). Twenty grams of the Roselle was placed in a stomacher bag and pulsified in 180 ml of distilled water for 15 seconds using a pulsifier (PUL 100E; Stuart Scientific Co. Ltd, U.K). From the stock solution, 1ml aliquots was inoculated into 10mls of prepared Buffered peptone water contained in a universal bottle and incubated for 24 hrs at 37^{o}C. This was followed by taking 1ml of the content of the universal bottle (the stock + peptone water) into 10ml of Selenite in triplicate and incubated for 48hrs at 37^{o}C. From each selenite broth tube, loopfuls were streaked on Salmonella-Shigella media (SS) and incubated at 37^{o}C for 48 hours. *Escherichia coli* was enumerated by transferring 1ml each of the positive tubes of Faecal coliforms into 5ml of tryptophan broth and incubated for 24hrs at 44^{o}C and then few drops of Kovacs reagent was added and a red ring meniscus indicated the presence of *Escherichia coli* and the MPN table was used for the counting.

3.4 DATA ANALYSIS

Laboratory data collected were analyzed using Analysis of Variance (ANOVA) using Statistix 9 software. Mean separation was done using Least Significant Difference (LSD) at P=0.05. Log transformation of the count was done using the formula [log10(x+1)], where the value 1 was added to each count (x) of thermotolerant coliforms, *Enterococci* and *E. coli.* in order to eliminate zero data

points. Mean data is however reported as untransformed values. Two-sample T-test was used to determine whether the mean values of fresh and dry Roselle leaves sold at the markets were statistically different from each other. Statistical significance was determined at P = 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 MICROBIAL QUALITY OF IRRIGATION WATER USED IN ROSELLE PRODUCTION IN THE KASSENA-NANKANA EAST MUNICIPALITY

Mean total coliform counts in the irrigation water sources were highest in the dam water followed by the well 3 water, canal and well 2. Total coliform counts from well 1 water were the lowest (Table 4.1). Statistically significant (P=0.0322) differences in total coliform counts were observed between dam water and canal, well 1, well 2 and well 3 water sources. However, water from the canal, well 1, well 2 and well 3 were not statistically (P>0.05) different.

Table 4.1: Total coliform numbers in irrigation water used in Roselle production

Irrigation water sources	Mean Count (cfu)	Log Count
(cfu) Dam	1.54×10^8	7.90
Canal	1.68×10^7	6.70
Well 1	9.69×10^6	6.80
Well 2	1.52×10^7	6.88
Well 3	2.17×10^7	6.91
$Lsd_{(0.05)}$	9.92×10^7	1.09
P-value	0.0322	0.1739
Cv%	151.58	10.29

Mean faecal coliform numbers followed a similar trend as seen for total coliforms

and were highest in the dam water followed by the canal, well 1 and well 3 (Table 4.2). Water samples from well 2 however were the lowest. Although there were differences in faecal coliform numbers between the dam, canal, well 1, well 2 and well 3 irrigation water sources, these were not statistically (P=0.5838) significant (Table 4.2).

Table 4.2: Faecal coliform numbers in irrigation water used in Roselle production

Irrigation water sources	Mean Count (cfu)	Log Count
(cfu) Dam	2.87×10^6	6.09
Canal	2.43×10^6	6.26
Well 1	2.15×10^6	6.21
Well 2	5.76×10^5	5.72
Well 3	1.04×10^6	5.80
$Lsd_{(0.05)}$	3.40×10^6	0.69
P-value	0.5838	0.3789
Cv%	124.47	7.64

Mean *Enterococci* numbers were also highest in the dam water followed by the canal, well 1 and well 2 and well 3 (Table 4.3). Statistically significant (P=0.0139) differences were observed in *Enterococci* counts between the dam irrigation water and well 1, well 2 and well 3 but between the dam and canal irrigation water sources, there were no significant differences and also between the canal, well 1, well 2 and well 3 (Table 4.3).

Table 4.3: *Enterococci* numbers in irrigation water used in Roselle production

Irrigation water sources	Mean Count (cfu)	Log Count (cfu)
Dam	252.5	2.40
Canal	217.5	2.33
Well 1	185.0	2.26
Well 2	185.0	2.27
Well 3	182.0	2.26
$Lsd_{(0.05)}$	43.38	0.08
P-value	0.0139	0.0108
Cv%	14.07	2.40

Mean *Escherichia coli* counts were highest in the dam water followed by well 3, well 1 and canal and well 2 (Table 4.4). Even though differences were observed in *Escherichia coli* count in the dam, canal, well 1, well 2 and well 3 irrigation water sources, these differences were not statistically significant (P=0.9418).

Table 4.4: *Escherichia coli* numbers in irrigation water used in Roselle production

Irrigation water sources	Mean Count (cfu)	Log Count
(cfu) Dam	6.85×10^5	5.81
Canal	5.55×10^5	5.72
Well 1	6.10×10^5	5.69
Well 2	5.55×10^5	5.72
Well 3	6.80×10^5	5.80
$Lsd_{(0.05)}$	4.46×10^5	0.35
P-value	0.9418	0.9239
Cv%	47.97	4.04

4.2 MICROBIAL QUALITY OF ROSELLE PRODUCED FROM DIFFERENT WATER SOURCES

Mean total coliform counts on the Roselle leaves cultivated using dam water recorded the highest counts, followed by that from well 2, canal and well 3 (Table 4.5). Roselle samples produced using well 1 water recorded the least total coliform count. Comparably, there were no statistically (P=0.6305) significant differences in total coliform counts on the Roselle leaves produced from the different water sources; dam, canal, well 1, well 2 and well 3 water sources (Table 4.5).

Contrastingly, faecal coliform counts on Roselle leaves were highest on that cultivated with well 2 followed by dam water, well 1 and well 3 (Table 4.6). Roselle leaves cultivated with canal water recorded the lowest. However, there were no statistically (P=0.5197) significant differences in the faecal coliform numbers irrespective of water source.

Enterococci counts were highest on Roselle leaves irrigated with canal water followed by dam water, well 1 and well 2 (Table 4.7). Roselle leaf samples from well 3 recorded the lowest *Enterococci* count. Differences in *Enterococci* counts on Roselle leaves produced from the different water sources were statistically (P=0.0806) different.

Escherichia coli counts on the other hand was highest on Roselle leaf samples irrigated from well 2 followed by canal and well 1. However, Roselle leaves irrigated with water from the dam and well 3 recorded the lowest *E. coli* counts (Table 4.8). Some differences in numbers on the Roselle leaves were observed depending on the irrigation water source used but these were not statistically significant.

Table 4.5: Total coliform numbers on Roselle produced from different water sources

Irrigation water sources	Count (cfu)	Log Count
(cfu) Dam	2.68×10^7	7.33
Canal	1.51×10^7	6.95
Well 1	9.81×10^6	6.80
Well 2	1.78×10^7	6.95
Well 3	1.19×10^7	6.94
$Lsd_{(0.05)}$	2.45×10^7	0.72
P-value	0.6305	0.6112
Cv%	99.89	6.87

Table 4.6: Faecal coliform numbers on Roselle produced from different water sources

Irrigation water sources	Count (cfu)	Log Count
(cfu) Dam	3.84×10^6	6.53
Canal	1.67×10^6	6.07
Well 1	3.35×10^6	6.50
Well 2	8.44×10^6	6.55
Well 3	2.17×10^6	6.23
$Lsd_{(0.05)}$	8.83×10^6	0.65
P-value	0.5197	0.4390
Cv%	150.54	6.73

Table 4.7: *Enterococci* numbers on Roselle produced from different water sources

Irrigation water sources	Count (cfu)	Log Count (cfu)
Dam	212.5	2.32
Canal	220.0	2.34
Well 1	172.5	2.23
Well 2	170.0	2.23
Well 3	165.0	2.22
$Lsd_{(0.05)}$	49.00	0.10
P-value	0.0806	0.0621
Cv%	17.29	2.93

Table 4.8: *Escherichia coli* numbers on Roselle produced from different water sources

Irrigation water sources	Count (cfu)	Log Count
(cfu) Dam	4.30×10^5	5.63
Canal	5.55×10^5	5.72
Well 1	5.55×10^5	5.72
Well 2	6.80×10^5	5.80
Well 3	4.30×10^5	5.63
$Lsd_{(0.05)}$	3.08×10^5	0.21
P-value	0.4146	0.4146
Cv%	38.51	2.40

4.3 MICROBIAL QUALITY OF ROSELLE LEAVES SOLD AT THE MARKET

Dry Roselle leaves sold on the market were slightly higher in total coliform numbers, compared to the fresh leaves but low in Enterococci and were the same in both dry and fresh for *Escherichia coli.* There were no statistically significant differences between microbial numbers on both the dry and fresh produce sold on the markets for total coliforms (P=0.4403), faecal coliforms (P=0.0585), *Enterococci* (P=0.3650) and *Escherichia coli* (Table 4.9, 4.10, 4.11 and 4.12).

Table 4.9: Two-sample T-test performed on total coliform numbers on fresh and dry Roselle leave samples sold at the market

Samples	Count (cfu)	Log Count (cfu)
Fresh Roselle	1.64×10^7	7.14
Dry Roselle	2.95×10^7	7.06
t-value	0.83	-0.19
P-value	0.4403	0.86

Table 4.10: Two-sample T-test performed on faecal coliform numbers on fresh and dry Roselle leave samples sold at the market

Samples	Count (cfu)	Log Count (cfu)
Fresh Roselle	3.36×10^6	6.50
Dry Roselle	1.26×10^6	6.02
t-value	-2.47	-2.69
P-value	0.0585	0.04

Table 4.11: Two-sample T-test performed on *Enterococci* numbers on fresh and dry Roselle leave samples sold at the market

Samples	Count (cfu)	Log Count (cfu)
Fresh Roselle	185.00	2.26
Dry Roselle	165.00	2.22
t-value	-0.98	-0.99

Table 4.12: Two-sample T-test performed on *Escherichia coli* numbers on fresh and dry Roselle leave samples sold at the market

Samples	Count (cfu)	Log Count (cfu)
Fresh Roselle	430.00	5.63
Dry Roselle	430.00	5.63
t-value	-	-

CHAPTER FIVE

DISCUSSION

5.1 MICROBIAL QUALITY OF IRRIGATION WATER USED IN ROSELLE PRODUCTION

The results obtained from the study indicated that the quality of irrigation water (canals, wells and dam) used in the production of Roselle leaves that is often eaten raw in the Kasena-Nankana municipality is poor. This is because the levels of total coliforms, faecal coliforms, Enterococci and *E. coli* were high in the dam, canals and wells. The poor microbial quality of the dam water source, which was the worse, might be due to contamination caused by human activities and livestock. This corroborates the findings of Harris *et al*, (2003); Jamieson *et al.* (2004) and Maciorowski *et al.* (2007) that, water source contamination originates from human activities and or animals, with the majority of these being of faecal origin or transferred through faeces. It is a common practice for people living around the dam areas to use the dam water for drinking, bathing, washing of clothes and for other water contact recreational activities such as swimming. Also wild and domestic animals that come to drink from these water sources often bath, defecate or urinate directly into the water.

The dam water seems to be more contaminated than that of the canal and well water. This could be due to the numerous anthropogenic activities that take place around the dam. Members of the community, aside bathing and washing of clothes in the dam, also use the dam water in moulding clay bricks for building. Secondly, there are no public toilets in the farm community and therefore farmers and other inhabitants

practice open defaecation which eventually get washed into the dams during the heavy rains when farming is also intense. Thirdly, because of the rich pastoral lands around the dam area, most animals graze along the dams contaminating it with their faeces. Fourthly, contamination of the dam water also comes from the intense agrochemicals and poultry manure use in farming.

The canal water being less polluted than the dam water could be due to receiving less pollutant from the environment due to the small surface area of the canal. It could also be that because the canal water is flowing water, the pollutants are distributed through advection and diffusion there by reducing the concentration of the pollutants as they flow from the nonpoint sources in to the canal. This agrees with Fei-Baffoe (2008) that, the effect of dilution and constant flushing action of the flowing water of the canal help in self-purification of canal water.

The well water being less polluted than the dam water could be that the wells received less pollutant because the water particles have to crawl through the pore spaces of the soil and the contaminants may easily be adsorbed on to the soil particles as the water passes through the soil in to the well when it rains (Fei-Baffoe,2008). Higher microbial indicator numbers have been recorded in shallow wells compared to that in streams in Kumasi, Ghana (Cornish *et al.*, 1999).This is because shallow wells or dugouts on vegetable farms were often not protected and could easily receive pollutants from the surrounding farm environment (Drechsel *et al.*, 2000; Amoah *et al.*, 2005; Obiri-Danso *et al.*, 2009). In most parts of the northern region, farmers unable to afford the cost of chemical fertilizers use cow dung as fertilizer in Roselle production and this could contribute to the contamination of the irrigation water sources. In the southern parts of the country however, most

vegetable farmers use fresh poultry litter for crop production which is also known to contaminate irrigation water (Drechsel *et al*., 2002).

Amponsah-Doku *et al*. (2010) and Drechsel *et al*. (2000) showed that farm run-offs could be a major source of contamination as it often carries faeces of wild birds, domestic animals, human excreta and household waste into water sources.The use of microbiologically contaminated irrigation water sources and produce handling practices increases the bacterial load on the Roselle crop (Mensah *et al*., 2001; Keraita *et al*., 2003; Amoah *et al*., 2005; Obiri-Danso *et al*., 2005). Amoah *et al*. (2005) showed that on farms where overhead irrigation techniques are used, larger leaf surface areas are exposed to the contamination from irrigation water and possibly from soil particles splashing unto the plant. Similarly, in this study where cow dung is applied as a major nutrient source, this could contribute to the high microbial indicator numbers (Lau and Ingham, 2001; Zschock *et al*., 2000).

Although it was expected that bacteria numbers would decrease from farm to market due to possible die-off resulting from the prevailing high temperatures and sunlight intensity, Roselle sold at the market showed higher levels of thermotolerant coliforms although *Enterococci* and *E. coli* counts were relatively low. This is because unlike coliforms, *Enterococci* are more sensitive to variations in environmental conditions and are easily knocked-off by sunlight and temperature (Obiri-Danso *et al*., 2001; Beuchat, 1998).The wide variations in environmental conditions at the market centres and the fact that Roselle leaves are exposed to high sunlight and temperature could account for the low levels of some of the microorganisms. According to Drechsel *et al*. (2000), the use of irrigation water in

washing vegetables at the farm gate after harvesting before transporting to the markets increases the bacterial load. Beuchat (1995) and Mensah *et al*. (2001) showed that poor vegetable handling practices, storage, transportation and cleaning practices at the market could add to the contamination.

CHAPTER SIX

6.1 CONCLUSION

The study indicated higher presence and number of microbial organisms in dam water as compared to well and canal water used in irrigating Roselle plant crops in the Kasena-Nankana municipality. Consequently Roselle leaf samples produced with dam water contain high indicator microorganisms. In addition, total coliform numbers on dry Roselle leaves sold at the market was higher than fresh leaves. Generally, quality of water for irrigation and production of Roselle leaves is very lower as compared to WHO and ICMSF standards.

Roselle, an important indigenous leafy vegetable, grown with dam water is therefore unsustainable as it is often high in microbial indicator organisms. However, the use of water from wells could be a safer option as microbial numbers are relatively low.

6.2 RECOMMENDATION

Based on the findings from the work conducted, the following recommendations are made.

1. Further research work should be carried out on the microbial quality of traditional leafy vegetables produced using animal manure.
2. For sustainable management of the water resource, the district assemblies should involve sanitation programmes to prevent pollution of water bodies and consequent transmission of water-related diseases since it is used for irrigation and other domestic uses.
3. Farmers should be assisted in fertilizer application by Agricultural Extension

Officers of the Ministry of Food and Agriculture to avoid possible contamination of surface waters used in irrigation.

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APPENDICES

APPENDIX 1: ANALYSIS OF VARIANCE (ANOVA) FOR WATER SOURCES

Completely Randomized AOV for Total_Col

Source	SS	MS	F	P
Treatm	6.106E+	1.526E+1	3.	0.0
Error	6.497E+	4.331E+1		
Total	1.260E+			

Grand Mean 4.34E+07 CV 151.58

Compl	Rando	AOV for		
Source	DF	SS	MS	F	P
Treatm		1.493E+	3.734E+12	0.	0.5
Error		7.647E+	5.098E+12		
Total		9.140E+			

Grand Mean 1.81E+06 CV 124.47

Completely Randomized AOV for Enterococ

Source	DF	SS	MS	F	P
Treatm		14870	3717.5	4.	0.0
Error		12425	828.33		
Total		27295			

Grand Mean 204.50 CV 14.07

Completely Randomized AOV for Escherich

Source	SS	MS	F	P
Treatm	6.532E+	1.633E+1	0.	0.9
Error	1.314E+	8.761E+1		
Total	1.379E+			

Grand Mean 617000 CV 47.97

APPENDIX 2: ANALYSIS OF VARIANCE (ANOVA) FOR WATER SOURCES (LOG TRANSFORMED)

Completely Randomized AOV for LogTC

Source	SS	MS	F	P
Treatm	3.852	0.9630	1.	0.1
Error	7.856	0.5237		
Total	11.70			

Grand Mean 7.0311 CV 10.29

Completely Randomized AOV for LogFC

Source	SS	MS	F	P
Treatm	0.955	0.2388	1.	0.3
Error	3.165	0.2110		
Total	4.121			

Grand Mean 6.0170 CV 7.64

Completely Randomized AOV for LogEntero

Source	DF	SS	MS	F	P
Treatm		0.058	0.0146	4.	0.0
Error		0.045	0.0030		
Total		0.104			

Grand Mean 2.3044 CV 2.40

Compl Randomized for

Source	DF	SS	MS		P
Treatm		0.047	0.0117	0.	0.9
Error		0.808	0.0539		
Total		0.856			

Grand Mean 5.7456 CV 4.04

APPENDIX 3: ANALYSIS OF VARIANCE (ANOVA) FOR ROSELLE LEAVES

Completely Randomized AOV for Total_Col

Source	SS	MS	F	P
Treatm	6.967E+	1.742E+1	0.	0.6
Error	3.971E+	2.647E+1		
Total	4.668E+			

Grand Mean 1.63E+07 CV 99.89

Completely Randomized AOV for Faecal_co

Source	SS	MS	F	P
Treatm	1.156E+	2.892E+1	0.	0.5
Error	5.150E+	3.433E+1		
Total	6.307E+			

Grand Mean 3.89E+06 CV 150.54

Completely Randomized AOV for Entericoc

Source	SS	MS	F	P
Treatm	10870	2717.5	2.	0.0
Error	15850	1056.6		
Total	26720			

Grand Mean 188.00 CV 17.29

Completely Randomized AOV for Escherich

Source	SS	MS	F	P
Treatm	1.750E+	4.375E+1	1.	0.4
Error	6.250E+	4.167E+1		
Total	8.000E+			

Grand Mean 530000 CV 38.51

APPENDIX 4: ANALYSIS OF VARIANCE (ANOVA) FOR ROSELLE LEAVES (LOG TRASFORMED)

Completely Randomized AOV for LogTC

Source	SS	MS	F	P
Treatm	0.634	0.1586	0.	0.6
Error	3.457	0.2305		
Total	4.092			

Grand Mean 6.9921 CV 6.87

Completely Randomized AOV for LogFC

Source	SS	MS	F	P
Treatm	0.734	0.1836	1.	0.4
Error	2.760	0.1840		
Total	3.494			

Grand Mean 6.3751 CV 6.73

Completely Randomized AOV for LogEntero

Source	SS	MS	F	P
Treatm	0.050	0.0125	2.	0.0
Error	0.066	0.0044		
Total	0.116			

Grand Mean 2.2670 CV 2.93

Completely Randomized AOV for LogEColi

Source	SS	MS	F	P
Treatm	0.078	0.0196	1.	0.4
Error	0.280	0.0187		
Total	0.359			

Grand Mean 5.7005 CV 2.40

APPENDIX 5: TWO-SAMPLE T TESTS FOR TOTAL_COLIFORM BY TREATMENT

Treatment	l	Mean	SD	SE
MARKET	4	2.95E+	3.01E+	1.50E
MARKET	4	1.64E+	9.78E+	4.89E
Difference		1.30E+	2.24E+	1.58E

T-Tests for Mean Difference

Null Hypothesis: difference = 0

Alternative Hyp: difference <> 0

					95% CI for Difference
Method	**Variances**	**DF**	**T**	**P**	**Lower**
	Upper				
Pooled	Equal	6	0.83	0.4403	-2.57E+07
	5.19E+07				
Satterthwaite	Unequal	3.6	0.83	0.4596	-3.28E+07
	5.89E+07				

Homogeneit	Varianc	D	F	P
Folded F Test		3,	9.	0.0

Cases Included 8 Missing Cases 0

APPENDIX 6: TWO-SAMPLE T TESTS FOR FAECAL_COLIFORM BY TREATMENT

Treatment	Mean	SD	SE
MARKET	1.26E+0	854004	427
MARKET	3.36E+0	1.47E+0	736
Difference	-	1.20E+0	850

T-Tests for Mean Difference

Null Hypothesis: difference = 0

Alternative Hyp: difference <> 0

Method	Variances	DF	T	P	95% CI for Difference Lower	Upper
Pooled	Equal	6	-2.47	0.0485	-4.18E+06	-19140
Satterthwaite	Unequal	4.8	-2.47	0.0585	-4.31E+06	111764

Homogeneit	Varianc	D	F	P
Folded F Test		3,	2.	0.1

Cases Included 8 Missing Cases 0

APPENDIX 7: TWO-SAMPLE T TESTS FOR ENTERICOCCI BY TREATMENT

Treatment	N	Mean	SD	SE
MARKET	4	165.00	17.321	8.66
MARKET	4	185.00	36.968	18.4
Difference		-20.000	28.868	20.4

T-Tests for Mean Difference

Null Hypothesis: difference = 0

Alternative Hyp: difference <> 0

Method	Variances	DF	T	P	95% CI for Difference Lower	Upper
Pooled	Equal	6	-0.98	0.3650	-69.947	29.947
Satterthwaite	Unequal	4.3	-0.98	0.3795	-75.352	35.352

Homogeneit	Varianc	D	F	P
Folded F Test		3,	4.	0.1

Cases Included 8 Missing Cases 0

APPENDIX 8: TWO-SAMPLE T TESTS FOR ESCHERICHIA COLI BY

TREATMENT	Treatment	N	Mean	SD	SE
M	D	4	430000	0.0000	0.00
M	F	4	430000	0.0000	0.00

ERROR: T-statistic is very large or very small.

ERROR: Data may be nearly constant or may need to be scaled.

Cases Included 8 Missing Cases 0