KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY (KNUST)

ASSESSMENT OF EFFECTIVENESS OF NON-TREATMENT INTERVENTIONS IN REDUCING HEALTH RISKS ASSOCIATED WITH CONSUMPTION OF WASTEWATER – IRRIGATED CABBAGE

By

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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ABSTRACT

Health risks associated with consumption of wastewater irrigated cabbage can be greatly reduced by treating the wastewater before its use. But wastewater treatment facilities are prohibitively expensive. The World Health Organization (WHO) in 2006 proposed alternative guidelines called non – treatment interventions for low – income countries for reducing health risk associated with wastewater irrigated vegetables. This study focused on the assessment of the effectiveness of non-treatment interventions in reducing potential health risks along production – consumption chain. The first phase of the study was a preliminary component that established the appropriate day for the cessation of irrigation before harvesting of cabbage, and post - harvest handling practices in the market and their contribution to the overall contamination. The study assessed post - harvest handling practices including places of display of cabbage during marketing, removal of outer leaves, and cutting of cabbage in the market for sales. Thermotolerant coliforms and helminth eggs levels were used as indicator organisms for health risk assessment. In this study, four-day cessation of irrigation before harvesting was identified as an acceptable on-farm non-treatment intervention. The four – day cessation of irrigation before harvesting could reduce thermotolerant coliforms by 0.83 log units and 0.6 helminth eggs per 100 g cabbage representing reduction rate of 0.21 log units and 0.15 helminth eggs per day. These reductions were both significant ($P \leq 0.05$). Removal of one outer leaf and display of cabbage on table covered with clean sack as intervention at the market reduced thermotolerant coliforms significantly by 0.97 log units ($P = 0.000$) and 0.2 helminth egg counts which was not significant ($P = 0.753$). Treatment of cut cabbage with vinegar reduced thermotolerant coliforms significantly by 2.11 $\log_{10}$ units and helminth eggs insignificantly (P = 0.909) by 0.6. Furthermore, treating cut cabbage with salt solution reduced both thermotolerant coliforms and helminth eggs significantly by 1.27 $\log_{10}$ units and 0.7 respectively. Overall cumulative reduction of 3.91 $\log_{10}$ units thermotolerant coliforms and 1.4 helminth eggs were achieved when cabbage pieces were treated with vinegar, and 3.07 log units thermotolerant coliforms and 1.5 helmith eggs for samples treated with salt solution. These figures are however, lower than the 6-7 log units' reduction proposed in the WHO guideline. The main species of helminth eggs isolated in the irrigation water and on the cabbage were *Ascaris lumbricoides*, *Schistosoma species, Strongyloides stercoralis, Taenia* species and *Trichuris trichiura.* The combinations of the non – treatment interventions have a potential of considerably reducing health risks associated with wastewater irrigated cabbage.

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DEDICATION

This work is dedicated to my wife Mary Tei and mum, Madam Eunice Agbo

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Wastewater use in agriculture is a common practice and is increasing as a result of the rising water scarcity worldwide (Scott *et al.*, 2004). In Ghana, urban vegetable farmers in search of irrigation water usually have no alternative than to use polluted waters to cultivate vegetables such as lettuce, spring onions, and cabbage which are usually consumed raw. However, the use of wastewater in cultivating these vegetables can pose a significant occupational and public health risk (Blumenthal and Peasey, 2002) to farmers and produce consumers (Smith *et al.*, 2003; Minhas *et al.*, 2006). The public health risks of using such contaminated streams or waters for irrigation have been widely discussed (Blumenthal *et al.*, 2000; Shuval et al., 1997; WHO, 2006).

Vegetables can become contaminated while growing, during harvesting, post-harvest handling or even during distribution and sale at the market (McMahon and Wilson, 2001). Many West African studies have reported high levels of pathogen contamination in irrigation water; and on both farm and market vegetables (Faruqui *et al.,* 2004; Amoah *et al.,* 2005; Niang, 1999) which far exceed the ICMSF standards (ICMSF, 1974). On farms, vegetables grown with untreated wastewater and poorly composted manures are more prone to microbiological contamination (Beuchat, 2002). In Ghana, high levels of faecal contamination have been reported in irrigation water and on vegetables grown in cities ranging between 3.00 and 8.30 $\log_{10}$ units per 100 gram faecal coliforms concentration (Amoah *et al.*, 2007a).

After harvesting, vegetable contamination occur from post-harvest handling practices and marketing activities. A study in Ghana by Amoah *et al*. (2007a) revealed that a lot of contamination occurs in markets though it does not significantly increase from farm through post-harvest handling and marketing. However, the study of Ensink *et al.* (2007) in Faisalabad, Pakistan showed that there was significant increase in *E. coli* and helminth eggs numbers in vegetables on the market harvested after 12 hours. Contaminations of fresh vegetables with pathogenic microorganisms may occur in homes due to cross-contamination (Zhao *et al.*, 1998). Market and street food survey in Accra estimated that every day, about 200 000 urban dwellers consume (fast) food containing raw vegetables produced with wastewater in urban and peri-urban agriculture (Obuobie *et al*., 2006; Amoah *et al*., 2007a). The consumption of this street food by urban poor has been cited as cause for high incidence of diarhoea diseases (King *et al*., 2000; Mensah *et al*., 1999).

Existing practices such as various irrigation application methods and the use of sanitizers in decontaminating vegetables before consumption have significant potential of reducing health risk. But these practices are being done in isolation at different points along producer – consumer chain, and not in combination to achieve a cumulative effect as have been proposed in the WHO (2006) guidelines.

The need to reduce the potential health risks resulting from faecal contamination of vegetables required a more holistic approach. This approach involves applying various multiple risk management practices along producer – consumer line.

This is referred to as the "multiple barrier approach" where barriers (non – treatment interventions or measures) are placed along the food chain to have aggregate or cumulative effects in reducing health risks as prescribed by the new WHO guidelines (WHO, 2006). WHO (2006) proposes measures such as wastewater treatment and other non-treatment interventions such as improvement on irrigation application techniques (that reduce direct contact of wastewater with the crop), crop restriction (which is useful and profitable to non - food crops) and cessation of irrigation before harvesting (which also allows natural pathogen die-off). Washing of produce with clean water and improved food hygiene reduce contamination at markets.

Better cooking practices, prevention of cross-contamination and effective pathogen decontamination processes, especially at food preparation points are all crucial components of a multiple barrier approach as proposed by the new WHO (2006) guidelines. Studies done in post-harvest treatments (washing and disinfection of vegetables after harvesting) emphasize the need for preventing contamination at all stages as post-harvesting treatment cannot eliminate pathogens without compromising the attractive and physical quality of produce (Beuchat *et al.*, 1998).

Some of these measures like irrigation methods, cessation before harvesting and improved sanitary washing methods have been tested on lettuce under local conditions in Ghana (Keraita *et al.,* 2007a,b, Amoah *et al.,* 2007b). The efficacies of these interventions on other vegetables which are also potentially consumed raw have not been assessed. In addition, these strategies have to be made locally appropriate so that they can easily be adopted by farmers and other stakeholders in the food chain to reduce potential health risks (Drechsel *et al.*, 2006).

1.2 Problem Statement

Health risks associated with wastewater reuse can be greatly reduced by treating the wastewater before its use. However, many of the existing wastewater treatment technologies are prohibitively expensive for low-income developing countries (Mariˇno and Boland, 1999). The WHO (1989) standards for intestinal nematodes (helminth eggs) and faecal coliforms in irrigation water are <1 egg / litre and <1000 faecal coliforms / 100 ml, respectively. Blumenthal *et al*., (2003) showed that low - income countries with treatment facilities cannot meet the requirements of the WHO (1989) guidelines. The enforcement of the guidelines in such situations would stop hundreds or thousands of farmers irrigating along polluted streams and put their livelihoods at risk. In view of these difficulties, the WHO (1989) guidelines needed adjustment for better application in wastewater exposed urban and peri-urban agriculture in resource-poor countries. Restrictions would also affect food traders and the general market supply with perishable crops, especially in cases where other water sources are (seasonally) unavailable.

Based on these difficulties, it was suggested during a consultation in Hyderabad, India, in November 2002 that WHO (1989) guidelines needed adjustment for better application in wastewater exposed urban and peri-urban agriculture in resource-poor countries (WHO, 1989). The new WHO (2006) guidelines are considered more flexible as they reduce the emphasis on previous microbiological standards as the basis of wastewater use in agriculture. In Ghana, some of the interventions on farm and at the kitchen level have been assessed for their efficiency in reducing contamination (Keraita *et al.*, 2007a, b, Amoah *et al.*, 2007b). But handling practices at markets which may increase contamination on vegetables are ill – defined. Because markets occupy a pivotal position in the supply of food, there is the need to identify adoptable non-treatment interventions at market level as well. To achieve the WHO (2006) recommendation of a performance target of 6-7 log units reduction, these proposed interventions have to be assessed from producer to consumer levels for their cumulative efficacy in reducing the pathogens concentration and the associated risks.

1.3 Research Questions

1. Do different post-harvest handling practices at the market significantly increase thermotolerant coliforms and helminth eggs numbers on cabbage?
2. Does cessation of irrigation before harvesting on farm significantly reduce thermotolerant coliforms and helminth eggs contamination of cabbage harvested?
3. Does the keeping of cabbage on clean sacks and on table with outermost leaf removed at market reduce thermotolerant coliforms and helminth eggs contamination significantly?
4. Does washing of cabbage with salt solution or vinegar at kitchens reduce thermotolerant coliforms and helminth eggs contamination significantly?

1.4. General Objective

To evaluate the efficiency of combinations of non-treatment interventions in reducing potential health risk associated with wastewater irrigated cabbage along producer – consumer pathway.

1.4.1 Specific Objectives

1. To determine the effects of different post - harvest handling practices on levels of thermotolerant coliforms and helminths on cabbage at market.
2. To determine the effectiveness of cessation of irrigation before harvesting in reducing thermotolerant coliforms and helminths levels on cabbage on - farm.
3. To determine the efficacy of sanitizers (vinegar or salt solution) in reducing thermotolerant coliforms and helminths levels on cabbage at street kitchen sites.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wastewater as a resource

Wastewater can both be a resource and a hazard. Wastewater has high potential for reuse in agriculture. This offers an opportunity for increasing food and environmental security, avoiding direct pollution of rivers, canals and surface water; conserving water and nutrients, thereby reducing the need for chemical fertilizer, and disposing of municipal wastewater in a low-cost, sanitary way (WHO, 2006).

The main sources of urban wastewater are domestic and industrial. Wastewater is mainly comprised of water, together with relatively small concentrations of suspended and dissolved organic and inorganic solids. Industrial wastewater is often associated with toxic elements such as heavy metals, but with limited industrial development in most developing countries, the greatest health concern when wastewater is used in agriculture is high levels of pathogenic organisms in untreated wastewater (Keraita *et al*., 2006).

Due to limited industrial development, domestic effluent and urban run-off contribute to the bulk of wastewater generated in Ghana. Domestic wastewater contains greywater (sullage), which is wastewater from washrooms, laundries, kitchens etc and may also contain blackwater, which is generated in toilets (Obuobie *et al*., 2006). Most developing countries lack proper infrastructure for safe handling of wastewater and much of it ends up in streams, rivers and irrigation canals, being partly used in farming (Keraita *et al*., 2006). In Ghana, less than 10% of urban dwellers are connected to piped sewerage system and wastewater is channeled from street gutters to large drains and inner – city streams (Keraita *et al*., 2003).

2.1.1 Wastewater Reuse in Urban Agriculture in Ghana

Urban and peri-urban agriculture (UA) is an activity that involves production, processing, and marketing of food and other products on land and water in urban and peri-urban areas. UA involves application of intensive production methods and (re)use of natural resources and urban waste to increase yields of a diversity of crops and livestock (UNDP, 1996).

This is an open-space vegetable farming which is carried out in both the rainy and the drying seasons. More than 15 kinds of vegetables are cultivated, all of which are sold. The most perishable (often non traditional) vegetables, such as lettuce, are usually grown and often harvested often during the year (with only supplementary irrigation during the dry season). The use of polluted water for vegetable farming is more widespread in the more populated cities where safe water is scarce and is used for domestic purposes. From a general survey among open-space farmers carried out in 2002, it was found that about 84% of nearly 800 farmers farming in and close to Accra and almost all 700 farmers in Tamale used polluted water for irrigation, at least during the dry seasons (Keraita and Drechsel, 2002).

Typical urban vegetable farm sizes range from 0.1– 0.2 ha and they increase in size along the urban–rural gradient. As production is market oriented, farming is input and output intensive, particularly in terms of the use of water and such other farm inputs as poultry manure, pesticides and fertilizers. In Ghana, most farmers use watering cans to irrigate (Keraita *et al*., 2003). Farmers fall into different age groups, but the majority are between 20 and 40 years old. Most of those engaged in urban agriculture are migrants from rural areas and have experience in farming.

2.1.2 Irrigated urban vegetable production in Kumasi

Kumasi is the capital town of Ashanti Region and the second largest city in Ghana with a population of one million and an annual growth rate of about 6 % (Ghana Statistical Services, 2000). Kumasi has a total area of 225 km^2 of which about 40% is an open land. Kumasi has a semi-humid tropical climate and lies in the tropical forest zone with an average annual rainfall of 1420 mm covering about 120 raining days. The rainfall pattern of the town is bimodal with the major season falling between the months of March and July and a minor rainy season around September and October. The mean monthly temperature of the area ranges from 24 to 27 °C. Important streams and rivers include the Owabi River, which flows through the suburbs of Anloga; Subin River, which passes through Kaasi and Ahensan; and Wiwi River, which runs through the KNUST campus (Obuobie *et al*., 2006).

In urban Kumasi, most land where farming is carried out belong to government institutions, private developers, etc. There are about 41 hectare of the urban area under vegetable irrigation while the peri-urban area has more than 12,000 hectare under irrigated vegetable farming mostly during the dry season (Cornish and Lawrence, 2001), twice as much as under formal irrigation in the whole country.

2.2 Cultivation of Cabbage *(Brassica spp.*)

Cabbage is from a group of plants known as the Cole crops. The word "Cole" means stem. Cole crops are from the family *Cruciferae*, a large family which contains many vegetables. It is also called the mustard family. The family name comes from the Latin word for "cross" and was given to members of this family because the flowers are cross shaped. Cabbage is one of the most common Cole crops, which thrives well in cool weather (Tiwari *et al*., 2003). It requires 60 to 100 days from sowing to reach market maturity depending on the variety (www.aces.edu). The optimum temperature range for cabbage production is 15 to 20 °C; growth stops when temperatures reaches above 25 °C. The minimum temperature is 0 °C (freezing), but cold hardened varieties can tolerate temperatures as low as -10 °C. Young plants less than six millimeters in diameter can tolerate both colder and warmer temperatures better than older plants (Bewick, 1994).

Poor environmental conditions during growth can lead to poor quality of the harvest product. High temperatures and low moisture can lead to low yields in cabbage. These conditions can also lead to long stems in the head and can cause the outer leaves to drop (Bewick, 1994). Higher temperatures can induce "bolting" in cabbage, but varieties differ in their susceptibility to this disorder. Bolting is the process in which the plant switches from vegetative growth (heading) to reproductive growth (formation of flowers and seeds). This switch becomes evident when seed stalks appear, making the heads unmarketable (www.aces.edu). The nutritional value of 100 g of edible portion contain 1.8 g protein, 0.1 fat, 4.6 g carbohydrate, 0.8 mg iron, 14.1 mg sodium, besides the enrichment in vitamins A and C (Tiwari *et al.*, 2003).

There is a large variety of cabbage cultivars available to growers. For fresh market, total yield is an important consideration, but quality considerations are foremost in cultivar selection. The size and the shape of the head are very important. Cabbage cultivars are classified by maturity, shape, leaf texture, and color. Cabbage can be grown on a wide range of soils (mineral, sand, and muck soils); but the crop is sensitive to soil acidity. The optimum pH is 6.0 to 6.5, and at pH greater than 7, the disease club root can be evident. Cabbage is a heavy user of nitrogen and potassium and requires frequent side dressing. Cabbage is considered a hard crop on the land, and many growers will rotate to other crops that do not have such high fertility requirements.

Irrigation is an essential element of a successful vegetable production operation and is critical to the consistent production of quality produce. Cabbage is a fast-growing, shallow-rooted crop whose roots penetrate only 12 to 15 inches into the soil. Although cabbage is relatively drought tolerant, adequate soil moisture levels should be maintained to maximize yields. In cabbage, the most critical period for irrigation is following direct seeding or transplanting and during head development. Any stress related to lack of water during these periods can lead to small head size (reduced yields), growth cracks, or tipburn. Any of these problems will result in loss in their marketability and value (www.aces.edu). Cabbage is irrigated by sub-surface irrigation (Bewick, 1994), and over head watering can irrigation in urban cities in developing countries (Keraita and Drechsel, 2002). On deeper sands it is a perfect crop for drip irrigation (Bewick, 1994). Regardless of the method used, cabbage requires about one inch of penetration of water in soil per week. The supply of water should be even throughout the growing season to prevent cracking of the heads (Bewick, 1994).

2.3 Potential health risks from using wastewater in agriculture

A number of risk factors associated with wastewaters use for agricultural irrigation have been identified. Some of these risk factors are short term and vary in severity depending on the potential for human, animal, or environmental contact (e.g. microbial pathogens, helminth eggs), while others have longer term impacts which increase with continued use of wastewater (e.g., saline effects on soil) (Toze, 2006).

The most common human microbial pathogens found in wastewater are enteric in origin. Enteric pathogens enter the environment through the faeces of infected hosts and can enter water either directly through defecation into water, contamination with sewage effluent or from run-off from soil and other land surfaces (Feachem *et al*., 1983). Wastewater usually contains high concentrations of varieties of these pathogenic organisms. The principal categories found in wastewater are viruses, bacteria, protozoa and helminths (Metcalf and Eddy, 1995; FAO, 1992). Infected human carriers of a particular disease may discharge these pathogenic organisms into the environment (Metcalf and Eddy, 1995). The numbers and types of pathogens found in wastewater vary both spatially and temporally depending on season, water use, economic status of the population, disease incidence in the population producing the wastewater, and quality of the water or food eaten. Animals may also discharge some pathogens such as bacteria in wastewater.

The risk of water-borne infection from any of these pathogens depend on a range of factors including pathogen numbers, dispersion in water, infective dose, chances of faecal contamination of the water and amount of treatment undertaken before potential exposure to the water (Haas *et al*., 1999). Table 2.0 shows the concentrations of major pathogens in typical raw wastewater.

Table 2.0: Excreted organism concentrations in wastewater

Organism	Numbers in wastewater (per litre)
Bacteria	
Thermotolerant coliforms	10^8–10^{10}
Campylobacter jejuni	10–10^4
Salmonella spp.	1–10^5
Shigella spp.	10–10^4
Vibrio cholerae	10^2–10^5
Helminths	
Ascaris lumbricoides	1–10^3
Ancylostoma/Necator	1 - 10^3
Trichuris trichiura	1- 10^2
Schistosoma mansoni	ND
Protozoa	
Cryptosporidium parvum	1–10^4
Entamoeba histolytica	1–10^2
Giardia intestinalis	10^2–10^5
Viruses	
Enteric viruses	10^5–10^6
Rotavirus	10^2–10^5

ND: no data. Sources: Feachem *et al*., (1983); Yates & Gerba, (1998).

2.3.1 Pathogens isolated or associated with vegetables

Spoilage bacteria, yeasts, and moulds dominate the microflora on vegetables, but the occasional presence of pathogenic bacteria, parasites, and viruses capable of causing human infections has also been documented (Beuchat, 1996). All types of produce have potential to harbour pathogens (Brackett, 1999) but *Shigella* spp., *Salmonella* spp., enterotoxigenic and enterohaemorrhagic *Escherichia coli*, *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum*, viruses, and parasites such as *Giardia lamblia*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* are of greatest public health concern (Beuchat, 1996). Vegetables can become contaminated with pathogenic microorganisms while growing in fields, orchards, vineyards, or greenhouses, or during harvesting, post-harvest handling, processing, distribution, and preparation in food service or home settings (Beuchat, 2002).

The greatest health risks are associated with crops eaten raw, such as salad crops, especially if they are root crops (e.g., radishes, onions) or grow close to the soil (lettuces, zucchinis). Some vegetables crops may be more susceptible to contamination than others, for example onions (Blumenthal *et al*., 2003), zucchini (Armon *et al*., 2002), and lettuce (Solomon *et al*., 2002). Generally, crops that have certain surface properties, e.g. hairy, sticky, crevices, rough, etc. protect pathogens from exposure to radiation and make them more difficult to wash off with rain or dislodge during post harvesting. The amount of water each crop traps also is also an important factor in the retention and survival of pathogens. For example, Shuval *et al*. (1997) estimated that lettuce retains 10.8 ml of irrigation water while cucumber holds only 0.36 ml - i.e. approximately three percent of the volume of water the lettuce retains. Stine (2005) showed that lettuce and cantaloupe surfaces retained pathogens from irrigation water spiked with *E. coli* and a bacteriophage (PRD1) but bell peppers, which have smooth surface did not.

2.3.1.1 Bacteria

Bacteria are the most common of the microbial pathogens found in wastewaters (Toze, 1999). There are a wide range of bacterial pathogens and opportunistic pathogens, which can be detected in wastewater. Many of the bacterial pathogens are enteric in origin. However, bacterial pathogens which cause non-enteric illnesses (e.g. *Legionella* spp., *Mycobacterium* spp., and *Leptospira*) have also been detected in wastewaters (Wilson and Fujioka, 1995; Fliermans, 1996; Neumann *et al*., 1997). Bacterial pathogens are metabolically active microorganisms capable of self-replication in the environment. In reality, however, these introduced pathogens are prevented from doing so by environmental conditions (Toze and Hanna, 2002). Like other enteric pathogens, a common mode of transmission is via contaminated water and food and by direct person to person contact (Haas *et al*., 1999). A number of these bacterial pathogens can also infect, or be carried by wild and domestic animals.

2.3.1.2 Thermotolerant Coliforms

The terms coliforms, thermotolerant coliforms and *Escherichia coli* are sometimes used in literature more or less interchangeably. Coliforms refer to the broad group of bile tolerant, gram negative bacteria that produce gas and acid from lactose at 35 to 37 °C. They occur naturally in raw water, soil, organic matter and faeces (Brenner *et al*., 1982). *Escherichia coli* and thermotolerant coliforms come from the family of bacteria known as *Enterobacteriaceae* (meaning 'family of bacteria living in the intestine'). *Escherichia coli* are nearly always present in the gut of humans and other warm-blooded animals. They are usually present in high numbers, and are found in fresh faecal matter at populations of more than 10^9 organisms per gram (Brenner *et al*., 1982). Thermotolerant coliforms and *Escherichia coli* are gram-negative facultative anaerobic bacilli that can ferment lactose at 44.5 ± 0.2 °C with the production of acid in 24 hours, in media containing bile salts (naturally found in the gut). Thermotolerant coliforms produce indole from tryptophan at 44.5 ± 0.2 °C and their presence indicate faecal contamination. Tests for thermotolerant coliforms can be simpler, but *Escherichia coli* are better indicator because some environmental coliforms (e.g. some *Klebsiella, Citrobacter,* and *Enterobacter*) are thermotolerant. *Escherichia coli* is the most common thermotolerant coliform present in faeces and is regarded as the most specific indicator of recent faecal contamination (Edberg *et al.*, 2000).

2.3.1.3 Helminth Eggs

The benefits of wastewater reuse may be limited by its potential health hazards associated with it. This is due to transmission of pathogenic organisms from the irrigated wastewater and soil to crops to grazing animals and humans (Korentajer, 1991). The majority of pathogens in wastewater are rapidly inactivated in the soil system (Reddy *et al.,* 1981), but some pathogenic parasites may survive in the environment for a long period. Protozoa and helminths found in wastewater are the parasites of primary public health when it comes to wastewater reuse. An important characteristic of these organisms is the production of a cyst or ova stage which aids their survival. Helminths are common in many areas and associated with poor hygienic practices. They produce ova and larvae aiding in their survival and dispersion in the environment (Amahmid *et al*., 1999).

Helminths are common intestinal parasites which are transmitted through the faecal-oral route (Toze, 1997). Some of these parasites require an intermediate host for development prior to becoming infectious for humans. These helminths that have complex life cycles requiring development in a secondary host are rarely a concern in wastewater reuse. Helminth parasites commonly detected in wastewaters that are of significant health risk in reused wastewaters include the round worm (*Ascaris lumbricoides*), the hook worm (*Ancylostoma duodenale or Necator americanus)*, and the whip worm (*Trichuris trichiura*). These helminths have a simple life cycle with no intermediate hosts and are capable of causing infection via the faecal-oral route (Toze, 1997). The presence of microbial pathogens in wastewater, particularly when sourced from sewage effluent is arguably the major concern for health regulators, farmers and the general public. One of the major sources of helminth infections around the world is the use of raw or partially-treated sewage effluent and sludge for the irrigation of food crops (WHO, 1989).

In Mexico, farmers, workers and their children who work in fields irrigated with untreated sewage effluent have been found to have a greater prevalence of round worm infection than the general population (Peasey *et al*., 2000). Studies show that infection rates, particularly for adults, decreased with treatment of the sewage effluent with infection rates decreasing at a rate that could be linked to the level of treatment. Peasey *et al*. (2000) also found that the consumption of raw vegetables (such as carrots, cauliflower, lettuce, and cucumber) irrigated with partially treated sewage effluent did not show any greater prevalence of infection from any age group to the general population. Public and commercial concern does exist regarding pathogens through the use of waste water and biosolids on cereal crops. It should be expected, however, that if there is a reduction of risk for the consumption of raw vegetables irrigated with partially treated effluent, then it can be surmised that grain crops irrigated with treated recycled waters would have even less risk from microbial pathogens. Even more specifically, grains are commonly processed further before they are consumed by humans, and which decrease the human health risk even further (WHO, 2006).

2.3.2 Factors affecting transmission of diseases from excreted pathogens

Most gastro-intestinal pathogens hardly reproduce outside the human or animal digestive tract. They have differing levels of resistance against die-off (i.e. survival times), depending on the environment in which they are contained (Table 2.1 and Table 2.2), and this affects the transmission of diseases (Keraita *et al*., 2006). Pathogens detection in wastewater, soil and on crops is an indication of potential health risks to populations consuming crops irrigated with wastewater and agricultural workers exposed occupationally. Indeed, wastewater may contain high concentrations of a wide variety of pathogens, and if used in an uncontrolled way, those reaching the field could survive long enough to constitute a potential disease transmission route. However, to actually cause disease a pathogen would have to survive the treatment processes or persist in the environment in sufficient numbers to infect a susceptible individual (Bastos, 1992). The helminths have the longest persistence in the environment. It is also clear that the survival time on crops is the least for all pathogens (Keraita *et al.*, 2006). Pathogens die or lose their infectivity in an exponential manner after they are excreted into the environment, irrespective of the type of environment, e.g. soil, crops and wastewater (Strauss, 1985).

Studies in this area have generally referred to a “safe period” as the time required (after wastewater irrigation is discontinued) to render the food crop safe for consumption. The “safe period” is determined by the die-off rate and number of surviving organisms which in turn depend on a number of factors, mainly environmental conditions (Vaz Da Costa Vargas, 1988).

Table 2.1: Factors which affect pathogen survival in the environment (WHO 2006)

Factor	Comment
Humidity	Humid environments favour pathogen survival Dry environments facilitate pathogen die-off
Soil structure	Clay soils and soils with high organic content favour survival of pathogens.
Temperature	Most important factor in pathogen die-off. High temperatures lead to rapid die-off; low temperatures lead to prolonged survival. Freezing temperatures can also cause pathogen die-off.
pH	Some viruses survive longer in lower pH soils, while alkaline soils are associated with more rapid die-off of viruses; neutral to slightly alkaline soils favour bacterial survival
Sunlight	Direct sunlight leads to rapid pathogen inactivation through desiccation and exposure to UV radiation
Foliage/plant type	Certain plants have sticky surfaces (e.g., zucchini) or can absorb pathogens from the environment (e.g., lettuce, sprouts) leading to prolonged survival of some pathogens; root crops such as onions are more prone to contamination and facilitate pathogen survival
Competition with native flora and fauna	Antagonistic effects from bacteria or algae may enhance die-off; bacteria may be preyed upon by protozoa

Table 2.2: Survival time of selected pathogens in different environments (in days unless otherwise stated)

Type of pathogen	In faeces, night soil and sludge	*In freshwater and sewage*	*In soils*	*On crops*
Viruses				
Enteroviruses	<100 but usually <20	<120 but usually <50	<100 but usually<20	<60 but usually <15
Bacteria				
Faecal coliform	<90 but usually <50	<60 but usually <30	<70 but usually <20	<30 but usually <15
Salmonella spp	<60 but usually <30	<60 but usually <30	<70 but usually <20	<30 but usually <15
Vibrio cholera	<30 but usually <5	<30 but usually <10	<20 but usually <10	<5 but usually <2
Protozoa				
Entamoeba histolytica	<30 but usually <15	<30 but usually <15	<20 but usually <10	<10 but usually <2
Helminths				
Ascaris lumbricoides	Many months	Many months	Many months	<60 but usually <30
Hookworm larvae			<90 but usually <30	<30 but usually <10
Taenia Eggs			Many months	<60 but usually <30
Trichuris trichura. Eggs			Many months	<60 but usually <30

Source: Feachem *et al.*, (1983)

2.4 Evidence of crop contamination

Wastewater containing pathogens can contaminate crops directly through contact during irrigation or indirectly as a result of soil contact, blowing dust, workers, and insects (Crook, 1998). Pathogens do not, however, readily penetrate fruits or vegetables unless the skin is broken. Contamination through roots is also minimal. Transmission of food-borne illness by enteric pathogens due to irrigation with wastewater has been established for more than 100 years (Yates and Garba, 1998) and this is the reason why irrigating crops, especially those eaten raw, with wastewater has been forbidden in some countries, such as Jordan (McCornick *et al*., 2004). However, most food-borne illness associated with enteric micro-organisms occurs during mishandling of food, typically when a sick food handler does not practice proper sanitation like hand washing (Martijn and Huibers, 2001). Epidemics due to contamination of crops in the field are hard to trace as contamination is likely to be more random and the food may be dispersed over a large geographic area. Association of illness with non-food crops is limited to people coming into contact with the wet parts of the crops in the field or during processing.

Pathogens generally survive less on crops than in soils, as sunlight exerts a lethal effect on all micro-organisms involved in contamination. As much as 99% elimination of detectable viruses has been reported after 2 days' exposure to sunlight, supporting regulations that a suitable time interval should be maintained between irrigation and crop handling or grazing time (Feigin *et al*., 1991). Nevertheless, crops can become contaminated at any time and the reliability and completeness of pathogen removal by mechanisms of desiccation, exposure to sunlight, starvation, etc. is questionable. In the case of food crops, the three best options for risk minimization are: (1) eliminating pathogens from wastewater before irrigation, (2) processing the crops before sale to the public, or (3) preventing direct contact between wastewater and edible portions of the crop (Crook, 1998). Post-harvest handling of crops, e.g. at markets and households, could even be more detrimental. This is influenced by culture and hygiene of the food and produce sellers as well as the consumers hence the need for more holistic approach to risk reduction has to extend to post-harvest handling.

2.4.1 Contamination along producer – consumer chain

The means by which pathogens contaminate fresh produce like vegetables are several. These include environmental sources in the field, contact with harvesting equipment and containers used to transport produce from the field to the market place, and perhaps in food service and home settings. Pathogens, along with spoilage microorganisms, may contaminate vegetables via several routes and at several points such as throughout the pre- and post harvest systems.

2.4.2 Pre-harvest contamination

Potential pre-harvest contamination sources of microorganisms include soil, faeces, irrigation water, inadequately composted manure, and human handling. Vegetables irrigated with wastewater have pathogens isolated on such crops after harvesting (Ensink *et al.*, 2007). Cross-contamination of produce with manure or improperly composted manure used on the farm can be a source of pathogen during pre-harvest. Crop contamination concerns leafy vegetables as every farmer broadcast the poultry manure over already established crops. During irrigation, the poultry manure is largely washed down. However, a study by Mensah *et al.* (2001) shows that farm gate samples of lettuce, cabbage and onions from poultry manure- treated fields contained high levels of total and faecal coliforms. A similar result was obtained by Amoah *et al*. (2005) who showed that on-farm crop contamination also takes place when piped water is used for irrigation. Sources of contamination in this case included the already contaminated soil (Faecal coliforms levels of 1×10^4 / 10 g in the upper 5 cm) and the frequent application of improperly composted poultry manure. Farmers and market salesmen also washed their produce in wastewater drains or wells on - farm.

2.4.3 Post-harvest contamination

Post-harvest sources include human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dust, transport vehicles, and processing equipment (Beuchat, 2002). Containers used to harvest, transport, and display vegetables are often not effectively cleaned and sanitized, and this can lead to contamination (Gabis and Faust, 1988). Single-use containers may hold produce for a sufficient time to allow the formation of biofilms of pathogens. Contamination of fresh produce with pathogens may result from contact with surfaces harbouring these biofilms. Pathogens attach to biofilms during transport or processing, enhanced their survival and growth (Dewanti and Wong, 1995). Growth of pathogens incorporated into biofilms would increase the probability of cross-contamination of produce.

A study by Ensink *et al*. (2007) showed that a significantly higher *Escherichia coli* and helminth concentrations were found on market produce; unsanitary post-harvest handling seems to have caused greater faecal contamination than wastewater irrigation.

This was confirmed by the presence of helminth eggs on cauliflower and smooth surface vegetables, which were free of helminth eggs at the field site. In Portugal, Vaz da Costa Vargas *et al*. (1996) attributed an increase in faecal bacteria concentrations from field to market to the use of contaminated water to refresh the produce at the market, a practice which is common in Ghana as well.

2.4.4 Contamination at street food kitchens

Ready-to-eat or ready- to-use vegetables are subjected to minimal or no processing before consumption. They can be used as ingredients in cooked dishes, but many are consumed raw without any treatment that would normally destroy pathogenic microorganisms (Thunberg *et al.*, 2001). In addition to contamination attributed to farming and harvesting practices, further handling in food service setting can increase levels of bacteria as well. Contaminations of fresh vegetables with pathogenic microorganisms also occur in homes due to cross-contamination (Zhao *et al.*, 1998). Chen *et al.* (2001) reported that even if proper hand-washing methods are followed, microorganisms may still be present and can be transferred from washed hands to vegetables during chopping.

2.5 Public health protection guidelines

To reduce health risks associated with wastewater use in agriculture, various guidelines and their revisions have been developed (WHO, 1989; 2006). These guidelines are based on epidemiological and microbial evidence from developed countries, which have disparate wastewater reuse systems with low - income countries (Blumenthal *et al.*, 2000). The WHO (1989) standards for intestinal nematodes (helminth eggs) and faecal coliforms in irrigation water are <1 egg / litre and <1000 faecal coliforms / 100 ml, respectively. These health guidelines are difficult to realize as sometimes even canal water supplies (e.g. in India) exceed these limits (Minhas *et al*., 2006). A study by Blumenthal *et al.* (2000) showed that low- income countries with treatment facilities cannot meet the requirements of the WHO (1989) guidelines. However, in many low – income countries, like Ghana, wastewater treatment as expected by the guidelines is not possible due to a variety of mostly economic reasons. The enforcement of the guidelines in such situations would stop hundreds or thousands of farmers from irrigating along polluted streams and put their livelihood at risk. Restrictions would also affect food traders and the general market supply with perishable crops, especially in cases where other water sources are (seasonally) unavailable. This led to the development of the new WHO (2006) guidelines, in which wastewater treatment is only one component.

The new guidelines also focused on health-based targets which could be achieved by various combinations of locally possible risk management options called non-treatment options or interventions. The new WHO guidelines proposed the use of a comprehensive risk assessment and risk management approach which encompasses all steps in the process, from generation and use of wastewater to produce consumption. This can be done by constructing multiple barriers along the process pathway by using various risk management strategies and interventions, so that they can have a combined resultant effect. For instance, to protect produce consumers, WHO (2006) proposed wastewater treatment, crop restrictions, use of application techniques that reduce produce contamination, prevention of cross contamination, improved food hygiene and better cooking of food. Effective pathogen decontamination processes, especially at the food preparation point are crucial components of the multiple barrier approach as proposed by the new WHO (2006) guidelines to minimize health risk associated with consumption of contaminated vegetables. These strategies have to be made locally appropriate so that they can easily be adopted by farmers and others in the food chain to reduce potential health risks (Drechsel *et al*., 2006).

2.5.1 Non-treatment interventions in reducing consumer health risks

The protection of public health can best be achieved by using a ‘multiple barriers’ approach that interrupts the flow of pathogens from the environment (wastewater, crops, soil etc.) to people. Human pathogens in the fields do not necessarily represent a health risk if other suitable health protection measures can be taken. Disability Adjusted Life Years (DALY) is the measure of the health of a population or burden of disease due to a specific disease or risk factor. DALY’s attempt is to measure the time lost because of disability or death from a disease compared with a long life free of disability in the absence of the disease. The health-based target of a tolerable additional burden of disease of $\leq 10^{-6}$ Disability Adjusted Life Years (DALY) per person per year (pppy) can be achieved, when treated wastewater is used for crop irrigation or by a combination of health-protection measures that produces an overall pathogen reduction of 6−7 log units as shown in Table 4.0 and Figure 1 (WHO, 2006).

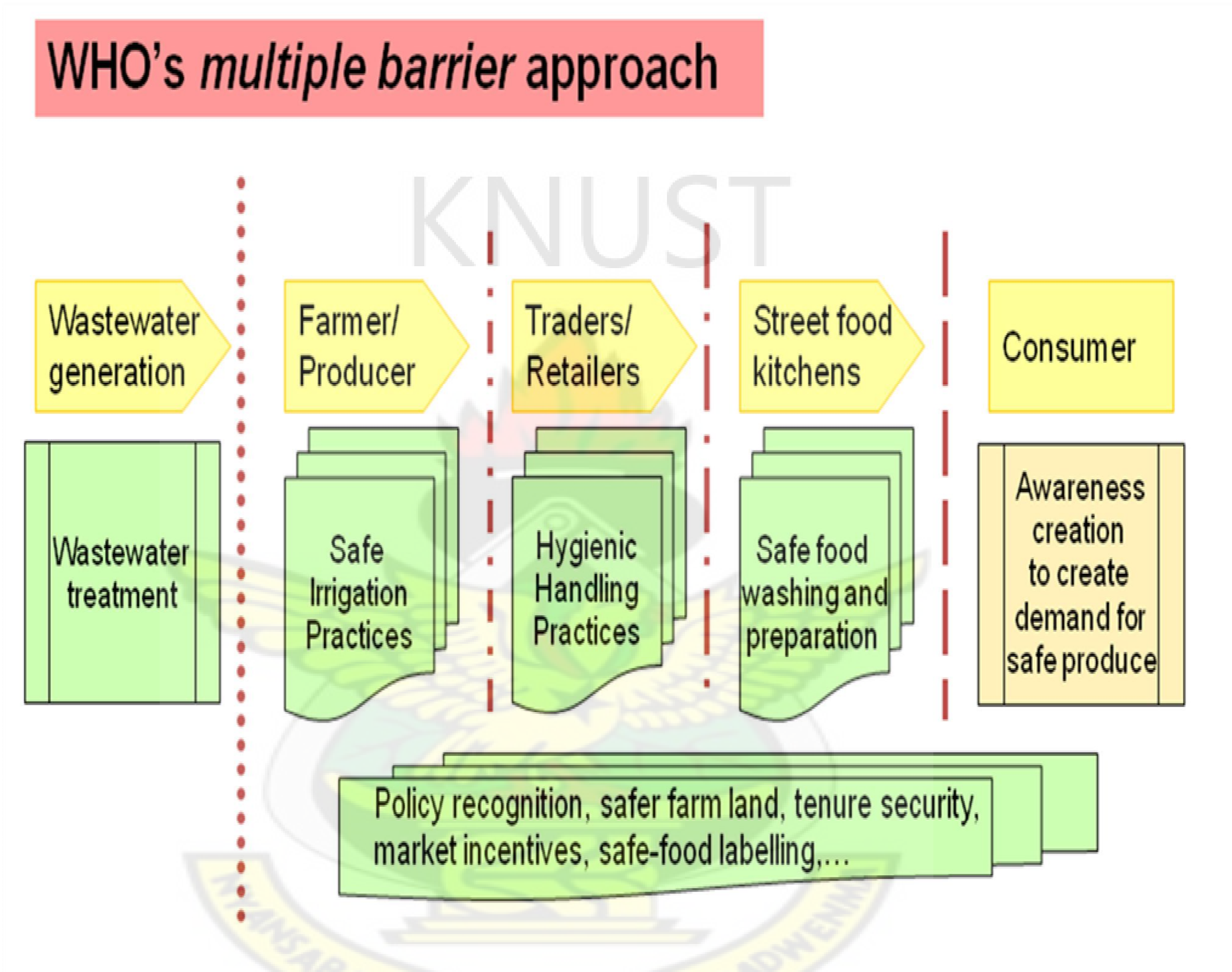

Figure 1: The multple barrier approch used in the study

Adapted from: WHO, 2006

Table 2.3: The effectiveness of selected health-protection measures that can be used to remove pathogens from wastewater irrigated crops

Protection measure (examples)	**Pathogen reduction (log units)**
Wastewater treatment (to different degrees)	1-6
Localized (drip) irrigation (with 'low-growing' crops, e.g. lettuce)	2
Localized (drip) irrigation (with 'high-growing' crops, e.g. tomatoes)	4
Pathogen die-off on the surface of crops after last irrigation	0.5-2 per day
Washing of produce with clean water	1
Disinfection of produce (using weak disinfectant solution)	2
Disinfection of produce (using one part vinegar on two parts of water)	5
Peeling of produce (fruits, root crops)	2
Cooking produce	6-7

Low growing crops: Root crops that grow just above, but partially in contact with the soil.
High growing crops: Crops that the harvested parts are not in contact with the soil.
Source*:* (WHO 2006).

2.5.1.1 Crop restriction

Restricted irrigation is recommended for many useful and profitable crops, including (a) non-food crops (e.g., cotton); (b) food crops that are processed before consumption (wheat); and (c) foods crops that have to be cooked (potatoes, rice). The choice of wastewater application method can impact on the health status of farm workers, consumers, and nearby communities. They included flood and furrow irrigation, spray and sprinkler irrigation, and localized irrigation. Localized irrigation techniques (e.g. bubbler, drip, and trickle) offer farm workers the most health protection because the wastewater is applied directly to the plants. Localized irrigation is estimated to provide an additional pathogen reduction of 2–4 log units, depending upon whether the harvested part of the crop is in contact with the ground or not.

2.5.1.2 Cessation of irrigation

Die-off on crop surfaces that occurs between last irrigation and consumption depends on climate (temperature, sunlight intensity, and humidity), time crop type, etc. A study conducted by Vaz da Costa Vargas *et al.* (1996) showed that cessation of irrigation with wastewater for 1–2 weeks prior to harvest can be effective in reducing crop contamination by providing time for pathogen die-off. However, enforcing withholding periods is likely to be difficult in unregulated circumstances because many vegetables (especially lettuce and other leafy vegetables) need watering nearly until harvest to increase their market value. However, it may be possible with some fodder crops that do not have to be harvested at the peak of their freshness (Blumenthal *et al.*, 2000). Alternatively, crops could be irrigated with other non-contaminated water sources (where available) after the cessation of wastewater use until harvest.

2.5.1.3 Pathogen die-off before consumption

The interval between final irrigation and consumption reduces pathogens (bacteria, protozoa, and viruses) numbers by approximately 1 log unit per day (Petterson and Ashbolt, 2003). The precise value depends on climatic conditions, with more rapid pathogen die-off (approximately 2 log units per day) in hot, dry weather and less per day in cool or wet weather without much direct sunlight (approximately 0.5 log unit per day). Helminth eggs can remain viable on crop surfaces for up to two months, although few survive beyond approximately 30 days (Strauss, 1996). The reduction potential die off rates should therefore be taken into account when selecting the combination of wastewater treatment and other health protection measures.

2.6 Handling practices at the market

Vegetable contamination may occur during production, harvesting and storage as well as during transport to the market before it finally arrives in the market. Because markets occupy a pivotal position in the supply of vegetables, as most vegetables pass through the market, markets afford the most cost-effective location for reducing further contamination of vegetables.

2.7 Food preparation measures

2.7.1 Washing

Vigorous washing of rough-surfaced salad crops (e.g. lettuce, parsley) and vegetables eaten uncooked in tap water reduces bacteria by at least 1 log unit; for smooth-surfaced salad crops (cucumbers, tomatoes) the reduction is approximately 2 log units (Brackett, 1999; Beuchat *et al*., 1998; Lang *et al*., 2004). Amoah *et al.* (2007a) showed that in Ghana most vegetables retailers especially lettuce wash their produce in the market before selling. Amoah *et al.* (2007b) also reported that washing vegetables irrespective of the method, and sanitizer type and concentrations commonly reduced faecal coliforms levels on lettuce. From their study, most of the common washing methods used in Ghana reduce faecal coliforms by a range of 1.0 - 2.2 log units with contact time of 2 min. A reduction of 0.2 -1.1 log units were observed when vegetables were just dipped into the washing solutions. The removal of helminth eggs requires first of all a physical process. Independently of the method or disinfectant used, washing in bowl reduced helminth egg population by half or more. Washing under a running tap (without any sanitizer) appeared even more effective, and could reduce helminth egg contamination levels from about 9 to 1 egg per 100 g wet weight (Amoah *et al*., 2007b).

2.7.2 Sanitizers or Disinfectants

In Ghana, salt solution, water and vinegar are mostly used for washing vegetables, whereas in the cities of her sister Francophone countries (Togo, Burkina Faso, Senegal, Benin etc), chlorine bleach ((commonly known as 'Eau de Javel') and potassium permanganate are well-known disinfectants (Amoah *et al*., 2007b). Salt is preferred to vinegar in Ghana because it is cheaper (Rheinla¨nder, 2006). Salt solution is a better sanitizer at an appropriate concentration of 35 ppm and 2 min contact time compared with potable water. Efficacy improves with increasing temperature and increasing concentration however, higher concentration has a deteriorating effect on the appearance of some crops such as lettuce. The use of vinegar reduced pathogens on cabbage by 0.5-0.9 log units between contact time of 5 - 10 min (Amoah *et al*., 2007b). Washing in a detergent (e.g., washing-up liquid) solution and rinsing in tap water can reduce helminth egg numbers by 1−2 log units (Jiménez-Cisneros, 2005).

2.7.3 Peeling off and cooking

Peeling fruits and root vegetables reduces pathogens by at least 2 log units. Cooking vegetables achieves an essentially complete reduction (5−6 log units) of pathogens.

These interventions are extremely reliable and should always be taken into account when selecting the combination of wastewater treatment and other health-based control measures. Effective hygiene education and promotion programmes will be required to inform local food handlers (in markets, in the home, and in restaurants and food kiosks) how and why they should wash wastewater-irrigated produce effectively with water or disinfectant and/or detergent solutions (WHO, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Kumasi, the second largest city in Ghana. It is located in the central part of the country. It has a population of 1.2 million, and an annual growth rate of 2.6% (Ghana Statistical Service, 2002). It covers a total area of 225 km^2 and the topography of the region varies from gently undulating to distinctly hilly and mountainous (www.maplandia.com).

The vegetation of Kumasi is the moist semi-deciduous forest type. It is characterized with an average annual rainfall of 1420 mm covering about 120 raining days. The main dry season, is from November to March. Daily minimum and maximum temperatures are 21.20°C and 35.50°C, respectively. The mean temperature is 28^o C. The relative humidity ranges between 75 to 79 % with average daily sunshine durations ranging between 2 and 7 hours (Meteorological Services Department, 2002).

In Kumasi, about 41 ha are cultivated with vegetables throughout the year. The production takes place on some 5–8 major open- space farming sites, usually along urban streams or in inland valleys with shallow hand dugout wells. Farming plots are owned by the government and private developers, and lack of tenure limits the investment farmers put into their plots (Keraita *et al.,* 2007a).

There are 3–5 larger markets and a significant number of community or neighborhood markets, often specialized in vegetables and fruits marketing in Kumasi (Amoah *et al.,* 2007b). Most of the cabbage sold in the markets originates from urban farms. In Kumasi, 95% of vegetables come from urban farms whiles 5% come from peri-urban farms. Rural farms contribute very little to supply of vegetables. Out of this 95%, 66% goes to the wholesalers. Vegetables are packed in sacks and transported in public buses or taxis to markets early in the morning or the previous night (Henseler *et al.*, 2005). Four vegetable markets in different locations were selected for the study. These markets are Racecourse, European, Ayigya, and Railways.

The farm trial was conducted at Ramseyer farming sites. Ramseyer farm is an urban vegetable production farm located at Gyinyase, a suburb of Kumasi. The farming land belongs to the Chief of Gyinyase. It is a low land area with a total land size of about 3.0 ha with 2.8 ha being irrigated under wastewater vegetable production. The average bed size is 40 m^2. Irrigation is done manually with watering cans being used in fetching water from shallow hand dugout wells at designated points on the farm.

The trials were conducted in the dry season, from November 2007 to February 2008 through farmers who agreed to denote their own plots for the participatory experimentation. Cabbage was selected for this study because cabbage is one of the vegetable often consumed raw and used in preparing salads. The average crop density at this site was one cabbage ball per m^2.

3.2 Research Methods

This first phase of the project was a preliminary study, which focused on assessment of cessation of irrigation before harvesting for cabbage as an on-farm intervention. This was done to establish the appropriate day for stopping irrigation before harvesting the cabbage. Assessment of post harvest handling practices at market such as places of displaying cabbage during marketing, removal of outer leaves of cabbage at market, effect of cutting cabbage at market, and washing of removed cabbage leaves (layer by layer) were carried out as part of the preliminary study.

3.2.1 Reducing contamination on-farm by cessation of irrigation before harvesting

3.2.1.1 Treatments and Field Design

Treatments were designed with 4-day intervals for up to 12 days of irrigation stoppage prior to harvesting. There were four treatments and as follows:

1. irrigating till harvesting day (T0)
2. stopping irrigation four days before (T4)
3. stopping irrigation eight days before harvesting (T8)
4. stopping irrigation twelve days before harvesting (T12)

Randomized complete block design (RCBD) was used for the arrangement of treatments. Four beds constituted one block. An average size of a bed was about 35 m^2. Each bed was divided into four sub plots of about 8.0 m^2. The four treatments were represented on each sub plot on a bed and were completely randomized on each of the two main blocks.

3.3 Sampling of cabbage from cessation of irrigation field trial

Samples were taken between 0600 and 0900 h GMT. Three cabbage ball samples were randomly cut from each sub plots (various treatments) into sterile polythene bags. Samples were transported in ice-cold box to the laboratory within 1 h for analysis. Two water samples were taken into sterilized 2–L bottles from each source used to irrigate all the beds during trial. Observations and informal interviews were carried out to document perceptions of participating farmers on wastewater–irrigated vegetable production.

3.3.1 Removal of outermost leaves on cabbage on- farm

In the laboratory, leaves were removed layer by layer and their proportions contribution to overall reduction of contamination determined. The initial weight of cabbage was determined and after each layer was removed, the weight of the remaining cabbage ball was recorded. Proportions of leaves removed were calculated from the initial weight and final weight after removal of leaves. The first sample constituted about 5-10 %; sample two, 15-20 %; sample three, 25-30 %; and sample four 30-45% of the total weight of cabbage. Three sub samples were taken from each layered sample set and analyzed for presence of thermotolerant coliforms and helminth eggs.

3.4 Observational study on handling practices at the markets

Observational study was carried out for three weeks by visiting each of the selected markets (Racecourse, Central, European and Ayigya) three times in a week, to observe vegetable handling practices. In addition, ten (10) of the sellers were informally interviewed to enable the researcher understand the rational behind some of the handling practices.

3.3.1 Quantification of effect of handling practices in the market: Based on observations made in the markets, an assessment study to quantify the effects of handling practices were carried out at only one of the markets (Racecourse) based on the following:

(1) **Display of vegetables:** During marketing, vegetable traders placed their cabbage at one of the three places: bare ground, on old sacks laid on the ground, and on tables. Three cabbages were randomly taken from each of the three display points and conveyed to the laboratory for microbiological analyses. In the laboratory, each set was cut into pieces and pooled into one. Three sub samples were taken from the pooled sample and analyzed for the presence of thermotolerant coliforms and helminth eggs.

(2) **Effect of removing outer cabbage leaves**: A set of cabbage from an identifiable farm was purchased at the market and used as the sampling unit. This was sub sampled for laboratory analysis as follows: (i) Single cabbage with one outer leaf removed by the traders in the market environment in the morning (0630 -0730 hrs) and displayed for sale and (ii) Single cabbage with outer leaves intact. Samples were taken at noon (1200 -1300 hrs) and then placed into cold ice box and conveyed to the laboratory for analyses for the presence of thermotolerant coliforms and helminth eggs.

(3) Effect of cutting cabbage at the market

Three single cabbages were purchased from three traders who sell already cut cabbage. The cut cabbage pieces were placed into sterile polythene bag. Another set of three cabbage samples were purchased and placed in sterile bag. The samples were transported on cold ice box to the laboratory. In the laboratory, three replicates were taken from the cut pieces bought from the market. The other cabbage set purchased was cut into pieces on cutting board wearing gloves and three replicates taken from this set too. Samples were analysed for the presence of thermotolerant coliforms and helminth eggs.

(4) Influence of market location on cabbage contamination

Three cabbages were purchased from each selected markets (Central, European and Ayigya markets). One outer leaf was removed from one set of the cabbage, and the other set was left intact. Samples were cut into pieces and analyzed for the presence of thermotolerant coliforms and helminth eggs.

(5) Washing of cabbage: Cabbage leaves were removed layer by layer. The layers were sampled by 10% weight and washed under running tap for 2 min. The washed leaves were separately cut into pieces and three sub samples taken for analysis. Unwashed cabbage balls with intact leaves serving as control were also cut into pieces and analyzed for thermotolerant coliforms and helminth eggs.

3.5 Assessment of non-treatment interventions in reducing faecal contamination of wastewater irrigated cabbage along producer – consumer pathway

3.5.1 Intervention I: Cessation of irrigation before harvesting

Cessation of irrigation with wastewater for some few days prior to harvesting can be effective in reducing crop contamination by providing time for pathogen die-off (WHO, 2006). From the first phase of the study, ceasing irrigation four days prior to harvesting was identified as the appropriate and feasible intervention. The four-day stoppage of irrigation prior to harvesting was selected in agreement with farmers who participated in the implementation of the trial. The experimental design of the trial was as follows: A bed with cabbage was divided into two sub plots. One sub plot was irrigated till harvesting (T0) while on the second sub plot irrigation ceased four days before harvesting (T4). Three cabbage samples were taken from each sub plots into sterile polythene bag in cold ice box for analysis in the laboratory. This trial was replicated at four different farm sites.

3.5.2 Intervention II: Removal of outermost leaf and display of cabbage on table covered with clean sack

From the preliminary study on removal of outer leaves, and display of cabbage for sale in the market, a combination of removal of one outer leaf of cabbage, and displaying cabbage on table covered with clean sack was used at the market. In this experiment, the traders who purchased the cabbage from the farm were followed from the farm gate to the market. On reaching the market, triplicate samples were obtained as follows:

a) Cabbage with the outermost leaf removed before displayed on table covered with clean sack and sampled at 0630:080 hrs

b) Cabbage with outer leaves intact was sampled on reaching the market at 600 hrs.

c) Cabbage sampled from the displayed batch (a) at 1200 -1300 hrs.

All samples were placed into sterile polythene bags in cold ice chest and conveyed to the laboratory for the determination of the presence thermotolerant coliforms and helminth eggs.

3.5.3 Intervention III: Washing with sanitizers at the street kitchen sites

Washing vegetables with sanitizers can reduce contamination levels. Amoah *et al*., (2007b) recommended the use of vinegar (1:5 $^{V}/v$) and salt solution of concentration of 35 parts per million (ppm) as inexpensive way for washing cabbage at the kitchen. Recommended vinegar concentration was used at the various street kitchens for this study, but salt solutions were prepared to suit the taste of food vendors (average concentration of 5 gram / litre).

The street kitchen vendor who purchased cabbage with one outer leaf removed and displayed on clean sacks on table at the market was followed to the kitchen, where the cabbages were used for salad preparation. At the kitchen, the food vendor was asked to cut the cabbage into pieces, using normal processes prior to sanitization and salad preparation. Subsamples of the cut cabbage were then further treated as follows: (i) washed in vinegar solution (1:5 $^{V}/v$) for 10 minutes and subsequently rinsed in cold tap water twice, (ii) washed in salt solution (35 ppm) for 10 minutes and rinsed in cold tap water twice, and (iii) no treatment with sanitizer. All treatments were done in triplicates and sub samples conveyed in a cold ice chest to the laboratory for the determination of the presence of thermotolerant coliforms and helminth eggs.

3.6 Laboratory analysis for thermotolerant coliforms and helminth eggs

3.6.1 Thermotolerant coliforms

The Most Probable Number (MPN) method was used to determine thermotolerant coliforms in all samples. Cabbage samples were aseptically cut into pieces and ten grams placed in a stomacher bag containing 90 ml of 0.9 % NaCl solution and then pulsified for 30 sec, using a pulsifier (PUL 100E 23003071, Microgen Bioproducts Ltd, UK). Dilutions of 10^{-1} to

10^{-11} were prepared by serially diluting 1 ml of the content of the stomacher bag. One millilitre aliquots from each of the dilutions were inoculated into 5 ml of MacConkey Broth with inverted Durham tubes and incubated at 44 °C for 18-24 hrs. Tubes showing colour change from purple to yellow and gas collected in the Durham tubes after 24 hrs were identified as positive. From each positive tube, a drop of the content was transferred into a 5 ml test tube of tryptone water and incubated at 44 ^{0}C for 24 hrs. A drop of Kovacs' reagent was then added to the tube of tryptone water culture. All tubes showing red ring colour development after gentle agitation denoted the presence of indole and were recorded as being presumptive positive for thermotolerant coliforms. Counts per 100 ml were calculated from Most Probable Number Tables (Anon, 1998).

3.6.2 Helminth eggs

Helminth eggs were enumerated using the concentration method (Schwartzbrod, 1998). This is a modified US-EPA method, but the same principle of floatation and sedimentation as in the method of Ayres and Mara (1996) was followed. About 100 g of cabbage leaves were thoroughly washed under running tap, and the washed water collected into a 2-L container and allowed to stand overnight to enable the eggs to settle completely. As much of the supernatant as possible was sucked and the sediment transferred into eight 50-ml centrifuge tubes. The 2-L containers were rinsed two to three times with tap water and the rinses were distributed into eight centrifuge tubes. The tubes containing the sediments were then centrifuged at 1450 rpm for 3 min. The supernatant was gently poured away and the deposit was re-suspended in about 150 ml $ZnSO_4$ solution (specific gravity = 1.2). The mixture was homogenized with a sterile spatula and centrifuged again at 1450 rpm for 3 min. The $ZnSO_4$ solution was added to cause the helminth eggs to float leaving other sediments at the bottom of the centrifuge tube. The $ZnSO_4$ supernatant (containing the eggs) was poured back into the 2-L container and diluted with at least 1L of distilled water. This was also allowed to stand for at least 3 h for the eggs to settle again. As much supernatant as possible was sucked and deposit was then transferred into eight centrifuge tubes. The 2-L container was rinsed two to three times with tap water and the rinsed water added to the centrifuge tubes and centrifuged at 1750 rpm for 3 min. The deposits were regrouped into one centrifuge tube and centrifuged at 1750 rpm for 3 min again. The deposit was re-suspended in 15 ml acid / alcohol buffer solution (5.16 ml 0.1N H_2SO_4 in 350 ml ethanol) and about 5 ml ethyl acetate was added. The mixture was shaken and the centrifuge tube occasionally opened to let out gas before centrifuging at 2200 rpm for 3 min. After the centrifugation, a diphasic solution (aqueous and lipophilic phase representing the acid ⁄ alcohol and ethyl acetate, respectively) was formed. With a micropipette, as much of the supernatant as possible (starting from the lipophilic and then the aqueous phase) was sucked out leaving about 1 ml of deposit. The deposit was placed on a microscope slide, observed under the microscope of × 40 magnification and the eggs counted. The eggs were identified by their shapes, sizes, and colour. The Bench Aid for the Diagnosis of Intestinal Parasites (WHO, 1994) was used for preliminary identification.

The number of eggs was calculated from the equation:

$N = AX/PV$

Where N = Number of eggs per sample

A = Number of eggs counted in the slide or mean counts from two or three slides

X = Volume of the final product (mL)

P = Volume of the slide (mL)

V = Original sample volume (L)

3.7 Data Analysis

Data were analyzed using SPSS for windows 13 (SPSS Inc, Chicago, IL, USA). Thermotolerant coliforms and helminth eggs counts were normalized by log transformation before analysis of variance (One-way ANOVA). Other data analysis, graphs and tables were obtained with Microsoft Excel Programme (Microsoft Corporation, 2003). Results of the analysis were quoted at $P \leq 0.05$ level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Irrigation water quality on - farm

The mean thermotolerant coliforms and helminth eggs counts in the irrigation water were estimated as 1.05×10^4 per 100 ml and 2.3 eggs / litre, respectively.

4.2 Contamination levels of cabbage on farm

Thermotolerant coliforms and helminth eggs counts decreased with an increase in the days of cessation of irrigation (Table 4.1).

Table 4.1: Influence of cessation of irrigation on mean numbers of thermotolerant coliforms and helminth eggs on cabbage

Cessation time (days)	No. of samples	Log_{10} MPN / 100g ± S.E Geometric mean	Helminth eggs per 100 g Arithmetic mean
0	18	5.66 (± 0.18)	2.0 (± 0.3)
4	18	4.69 (± 0.12)	1.6 (± 0.2)
8	18	3.93 (± 0.13)	1.5 (± 0.2)
12	18	3.25 (± 0.12)	1.4 (± 0.2)

Figures in parentheses are standard errors. (n = 72)

Stoppage days before harvesting significantly influenced population of thermotolerant coliforms (P = 0.00), but the differences in helminth eggs counts were not significantly (P=0.129) affected (Appendix 1). Multiple comparison analysis showed that thermotolerant coliforms numbers on cabbages harvested after four days cessation of irrigation does not differ significantly from that of eight days cessation treatment. But the difference between mean thermotolerant coliform counts on cabbages harvested from four days and that of twelve days cessation period were statistically significant (Appendix 1A). Contrast analysis between the control (irrigation till harvesting – T0) and other treatments (different cessation days) also showed significant difference (P < 0.05) for both thermotolerant coliforms and helminth eggs counts (Appendix 1B).

4.2.1 Effect of outer leaves removal on decontamination

Higher counts of thermotolerant coliforms and helminths were recorded on outermost leaves that were in direct contact with the environment (especially soil). The deeper the leaves were removed towards the core of the cabbage, lower the thermotolerant coliforms and helminths egg counts. Statistically analysis showed that the difference observed were significant (P = 0.000) as shown in (Table 4.2) (Appendix 2).

Table 4.2: Effect of outer leaf removal on decontamination of cabbage

Outer leaves removed		Log_{10} MPN / 100g ± S.E	Helminth eggs per 100 g
Number of layers	Proportion of weight (%)	Geometric mean	Arithmetic mean
0	0	5.66 (± 0.15)	2.4 (± 0.3)
1	10	4.67 (± 0.12)	1.5 (± 0.2)
2	20	3.82 (± 0.19)	0.8 (± 0.1)
3	30	1.24 (± 0.62)	0.0 (± 0.0)
4	40	0.0 (± 0.00)	0.0 (± 0.0)

Figures in parentheses are standard errors. (n = 45).

4.3 Observational study on post-harvest handling practices in the market

This study was carried out to identify the different handling practices associated with sale of cabbage in the market. The study revealed key handling practices in the various markets, as follows: different modes of displaying vegetables, removal of outer leaves prior to sale, and cutting of cabbage for customers in the market. Informal interviews carried out revealed more insight on marketers' reasons for adopting such handling practices. Traders who often displayed cabbages on the bare ground were wholesalers. This practice was carried out to facilitate sorting out the cabbage into different sizes for retailing. Few traders displayed their cabbages on old sacks on the ground. Traders who displayed cabbage on table had permanent stalls in the markets; whiles those who display their wares on the ground did not have permanent selling points. The removed outer leaves are often the unsightly ones. The removal of the outer leaves is done to expose the fresh inner part of cabbage balls to make it more attractive to customers. Cutting of cabbage at the market is done on request by the customer. Most of traders were unaware that their handling practices contribute to contamination of cabbage.

4.4 Handling practice and contamination of cabbage in the markets

Vegetables displayed on bare ground had higher levels of thermotolerant coliforms and helminth eggs than those placed on raised tables (Table 4.3). There were slight differences ($P \geq 0.05$) in the numbers obtained for both thermotolerant coliforms and helminth eggs. Cabbages displayed on the ground had a population of $\log_{10}$ 6.37 to $\log_{10}$ 9.62 thermotolerant coliforms and helminth eggs of 0.6 to 3.8 per 100 g. Thermotolerant coliforms population ranged from $\log_{10}$ 6.18 to $\log_{10}$ 9.62 and from 0 to 3.1 helminth eggs per 100 g of samples from ground covered with old sack. Cabbage samples from tables covered with clean sack had thermotolerant coliform counts ranging from $\log_{10}$ 5.18 to 8.96 and from 0.5 to 3.1 helminth eggs per 100 g. These differences were however, not statistically significant for both thermotolerant coliforms *(P* = 0.422) and helminth eggs counts *(P* = 0.578) (Appendix 3).

4.4.1 Influence from market classes environment

The handling practices at the various market places assessed depended on the socioeconomic class of people who patronize the goods at the market (i.e. sellers and consumers). Handling practices were different in each market. An estimated of 20% percentage traders in all the markets removed the highly contaminated outermost leaves. In all the markets, higher contamination levels were obtained on cabbage with intact outer leaves (Table 4.4). European market cabbage samples had the lowest levels of contamination on intact outer leaves compared to those of Ayigya and Central markets. Ayigya market had the lowest contamination levels on the overlying layer when outer leaves were removed compared to European and Central Markets. Generally, an average reduction of 1.24 $\log_{10}$ thermotolerant coliforms and 0.7 helminth eggs per 100 g wet weight were obtained when outer leaves of cabbage were removed

Table 4.3: Contamination levels of cabbage sampled from three display points in the market

Displayed point	No. of samples	Log_{10} MPN / 100g ± S.E	Helminth eggs per 100 g
		Geometric mean	Arithmetic mean
Bare Ground	9	7.75 (± 0.35)	1.8 (± 0.3)
Ground covered with old sack	9	7.61 (± 0.36)	1.3 (± 0.3)
Table covered with sack	9	6.98 (± 0.47)	1.4 (± 0.3)

Figures in parentheses are standard errors. (n = 27)

Table 4.4 : Influence of different market environments and handling practices on cabbage contamination

Market	Handling Practices	No. of samples	Log_{10} MPN / 100g ± S.E	Helminth eggs counts per 100 g
			Geometric mean	Arithmetic mean
Ayigya	Outer leaves removed	8	4.92 (± 1.42)	0.9 (± 0.7)
	No outer leaves removed	8	6.57 (± 1.90)	1.9 (± 0.8)
European	Outer leaves removed	8	5.13 (± 0.77)	1.3 (± 0.5)
	No outer leaves removed	8	6.31 (± 0.38)	1.7 (± 0.7)
Central	Outer leaves removed	8	5.65 (± 0.46)	1.3 (± 1.0)
	No outer leaves removed	8	6.84 (± 0.69)	2.0 (± 1.0)

Figures in parentheses are standard errors (n = 48).

4.4.2 Removal of outermost leaves and cutting of cabbage at the market prior to sale

Cabbage is normally sold in the various markets with outer leaves intact. Cabbage samples with outer leaves intact had geometric mean thermotolerant coliforms and mean helminth eggs of 7.07 $\log_{10}$ units and 3.1 eggs per 100 g respectively, whiles those with outer leaves removed had geometric mean of 5.97 $\log_{10}$ and mean 1.5 helminth eggs counts per 100 g. Removal of outer leaves reduced thermotolerant coliforms by 1.1 log units per 100 g wet weight, whereas helminth egg number was reduced by 1.6 eggs per 100 g wet weight. This reduction was significant for thermotolerant coliforms (P = 0.000), but not for helminth eggs (P = 0.118) (Appendix 4).

The cutting place (environment) of cabbage for food preparation also has influence on contamination levels. Cut cabbage pieces obtained from the market had geometric mean thermotolerant coliform level of 6.93 $\log_{10}$ units and mean helminth eggs of 2.2 counts per 100 g, but those prepared in the laboratory had geometric mean thermotolerant coliforms of 4.03 $\log_{10}$ units and mean helminth eggs of 1.9 per 100 g. This difference was significant for thermotolerant coliforms (P = 0.001), but not for helminth egg counts (P = 0.164) (Appendix 5).

4.5 Handling practices at the street kitchen sites

Unwashed cut cabbage pieces with running tap had a geometric mean thermotolerant coliforms of 6.95 $\log_{10}$ units and 2.2 helminth counts per 100 g, whiles washed samples had 3.96 $\log_{10}$ units and 0.6 helminth counts per 100 g. It was observed that washing under running tap for two minutes reduced thermotolerant coliforms counts by 2.97 $\log_{10}$ units per 100 g and helminth egg counts by 1.6 per 100 g. These reductions were significant (P = 0.000) for both thermotolerant coliforms and helminth eggs (Appendix 6).

4.5.1 Effectiveness of sanitizers in decontamination of cabbage

Washing of cabbage with vinegar or salt solution reduced contamination levels on the cabbage (Table 4.5). Significant log reductions of thermotolerant coliforms were obtained for both the vinegar (P = 0.000) and salt solution (P = 0.000). However, helminth eggs reduction was significant (P = 0.019) when salt solution was used, but not significant (P = 0.909) with vinegar (Appendices 7 and 7A).

Table 4.5: Influence of sanitizing practice on the reduction of thermotolerant coliforms and helminth eggs resident on cabbage.

Washing method	No. of samples	Log_{10} MPN / 100g ± S.E (Geometric mean)		Helminth eggs 100^{-1} g (Arithmetic mean)	
		Initial	Final	Initial	Final
Vinegar	21	3.86^{a} (± 0.08)	1.75^{b} (± 0.03)	0.7^{a} (± 0.05)	0.1^{a} (± 0.08)
Salt solution	21	3.92^{a} (± 0.08)	2.65^{b} (± 0.31)	1.1^{a} (± 0.1)	0.4^{b}(± 0.09)

Figures in parentheses are standard errors; (n = 42).
Initial: Pathogen level before interventions
Final: Pathogen level after intervention
Mean values of thermotolerant coliforms or helminth eggs for a given sanitizer followed by the same letter are not significantly different at $P \leq 0.05$.

4.6 Cumulative effectiveness of the selected non-treatment interventions along the production – consumption pathway

After the assessment of the various non-treatment interventions individually at the different entry points (i.e. farm, market and street kitchen) along the producer – consumer chain, the best risk reducing interventions were combined and their cumulative effectiveness assessed. In this study, the multiple barrier approach to interrupt pathogen flow proposed by the WHO (2006) was employed. The methods included cessation of irrigation before harvesting on farm, removal of outermost leaves of cabbage and displaying vegetable on table covered with clean sack at the market, and washing cut cabbage pieces with vinegar or salt solution at the kitchen.

Cessation of irrigation before harvesting on farm reduced thermotolerant coliforms by 0.83 $\log_{10}$ units for the four-day stoppage intervention representing reduction rate of 0.21 log units per day , while 0.6 eggs per four days reduction was achieved for helminth eggs (Table 4.6). This reduction was statistically significant for both thermotolerant coliforms and helminth egg counts ($P \leq 0.05$) (Appendix 8). The removal of one outer leaf and display of cabbage on table covered with clean sack for sale imposed on this same batch of cabbage at the market reduced thermotolerant coliforms by 0.97 log units significantly (P = 0.000) and helminths by 0.2 eggs which was not significant (P = 0.735) (Appendix 8A). In the street kitchen, washing of the same batch of cabbage with vinegar significantly resulted in a further 2.11 $\log_{10}$ reduction of thermotolerant coliforms (P = 0.000) and not significant 0.6 reduction of helminth eggs (P = 0.909). Washing a batch of cabbage with salt solution resulted in 1.27 $\log_{10}$

reductions of thermotolerant coliforms and 0.7 helminth eggs. These reductions were statistically significant ($P \leq 0.000$) (Appendices 7 &7A).

Table 4.6 Influence of the indicated treatment effects on the reduction of thermotolerant coliform and helminth eggs resident in cabbage

	Log_{10}Thermotolerant coliforms / 100 g (± S.E)			Helminth eggs / 100 g		
Interventions	*Initial*	*Final*	*Reduction*	*Initial*	*Final*	*Reduction*
Cessation of irrigation for 4 days before harvesting	5.64 ^{a} (±0.10)	4.81^{b} (±0.08)	0.83	2.1 ^{a} (±0.2)	1.5 ^{b} (±0.1)	0.6
Removal of one outer leaf and display of cabbage on table covered with clean sack for sale	4.88 ^{a} (±0.10)	3.91^{b} (±0.85)	0.97	1.3 ^{a} (±0.2)	1.1 ^{a} (±0.2)	0.2
Cabbage washing with vinegar for 10 minutes	3.86 ^{a} (±0.08)	1.75 ^{b} (±0.03)	2.11	0.7 ^{a} (±0.08)	0.1 ^{a} (±0.05)	0.6
Cabbage washing with salt solution for 10 minutes	3.92 ^{a} (±0.14)	2.65 ^{b} (±0.31)	1.27	1.1 ^{a} (±0.1)	0.4 ^{b} (±0.09)	0.7

Figures in parentheses are standard errors; (n = 84)
Initial: Pathogen level before intervention;
Final: Pathogen level after intervention.
Mean values of thermotolerant coliforms or helminth eggs for a given intervention followed by the same letter are not significantly different at $P \leq 0.05$

The reduction obtained by cessation of irrigation before harvesting is lower than the range proposed in the WHO guideline (Table 4.7). The use of vinegar marginally reduced contamination levels above the proposed reduction range whiles reduction obtained from salt solution was below values reported in the Guidelines. A cumulative reduction of 3.91 $\log_{10}$ units of thermotolerant coliforms and 1.4 helminth eggs were achieved when the cabbage pieces were washed in vinegar. However, washing in salt solution gave cumulative reduction of 3.07 $\log_{10}$ units and 1.5 helminth eggs respectively.

Table 4.7: Thermotolerant coliforms reduction achievable by various interventions in comparison with those reported by WHO (2006)

Interventions	$\log_{10}$ Thermotolerant coliforms / 100g Reduction		Helminth eggs No. / 100g Reduction
	*WHO	This study	This study
Cessation for 4 days before harvesting	0.5 – 2 per day	0.21 per day	0.6
Display of cabbage on table covered with clean sack and outermost leaves removed	NA	0.97	0.2
Washing cabbage with vinegar for 10 minutes	2.0	2.11	0.6
washing cabbage with salt solution for 10 minutes	2.0	1.27	0.7

Thermotolerant coliforms reduction levels in the proposed *WHO guidelines (2006).
NA: Not available.
No reduction of helminth eggs available with the proposed interventions are in the WHO (2006) guidelines.

4.7 Types of helminth eggs identified:

(a) Irrigation water

Different types of helminth eggs / larvae were identified in the irrigation water as well as the cabbage (Tables 4.8 and 4.9). The different eggs were identified using the Bench Aid reference as follows: the eggs with yellow to brown conspicuous colour, decorticated or mammilated outer shell as revealed by the light microscope suggested that they were *Ascaris lumbricoides* eggs (Appendix 9). Those eggs which appeared thin, elongated with transparent shell and prominent lateral spine near the posterior end were identified as those of *Schistosoma* spp. Larvae that appeared long and slim in nature with small opening at the lower end and a prominent tail were identified as *Stongyloides stercoralis.*

(b) On Cabbage samples

A total mean count of 39.5 helminth eggs population types were identified on the cabbage at the various sampling points during the study period (Table 4.9). Cabbage samples obtained from the markets had the highest helminth eggs counts with the least counts obtained on cabbages treated with sanitizers at the kitchen. Morphological characteristics observed which aid in identification were the same described above suggested that the different eggs types obtained included *Ascaris lumbricoides, Schistosoma species,* and *Strongyloides stercoralis*. For *Taenia* species, there were identified using their thick shell egg which contained a six hooked embryo. Some of the eggs appeared larger and swollen with less sharply defined bipolar ends and the layers of the shell under the microscope, and these represented *Trichuris trichiura*. *Ascaris* *lumbricoides* were the highest helminth eggs type observed on the cabbage, while *Taenia* species were the least identified type.

Table 4.8: Types of helminth eggs identified in the irrigation water

Types of helminth eggs identified	**Percentage of helminth eggs per litre**
Ascaris lumbricoides	52.2
Schistosoma species	39.1
Strongyloides stercoralis *[*]	8.7
Total helminth eggs	100

*These are larvae and not eggs (n = 10)

Table 4.9: Types of helminth eggs on cabbage sampled from farm, market and street kitchen

Types of helminth eggs identified	**Mean number of helminth eggs per 100 g**			
	Farm	**Market**	**Street kitchen**	**Total**
Ascaris lumbricoides	11.4	7.8	1.4	20.6
Schistosoma species	4.8	4.2	0.7	9.7
Strongyloides stercoralis *	0.5	6.0	-	6.5
Taenia species	0.4	-	-	0.4
Trichuris trichiura	-	2.3	-	2.3
Total helminth eggs	17.1	20.3	2.1	39.5

*These are larvae not eggs (n = 369)

4.8 Perception of various stakeholders along the producer-consumer chain

4.5.1 Farmers' perception

Farmers who carried out the on-farm intervention (cessation before harvesting) trial stated that adoption of this intervention is feasible. From the informal interview carried out, most farmers normally leave the cabbage on their beds after maturing while looking for customers. According to them, the quality of cabbage would not deteriorate provided it was well matured. However, during the study period, it was observed that outer leaves deteriorated, but these were normally removed on the farm before the cabbage was sent to the market or these leaves were removed at the market. Farmers considered cessation of irrigation before harvesting as an easy and affordable means of reducing surface contamination of cabbage leaves.

4.8.2 Perception of traders on removal of one outermost leaf and displaying of cabbage on clean sack on a table for sale

Interaction with traders at the market revealed that the acceptance of the intervention depended on the sellers' category. Wholesalers have their regular customers (retailers) did not accept removal of outer leaves. Wholesalers said removal of outer leaves would reduce the size and consequently the price of the cabbage. But some retailers welcomed the idea of removing outer leaves because it attracted customers when the unsightly outermost leaves were removed. This was already being done by most retailers. Retailers that have permanent stalls in the markets often display their produce on tables covered with clean polythene sacks, whiles sellers who display their cabbage on the ground were mostly temporary wholesalers who store produce for relatively short period of time. This market intervention of removing outermost leaves and displaying cabbage on table covered with sack could easily be adopted by some retailers who sell directly to vegetable consumers. Some of the retailers were skeptical about adopting this intervention because of potential loss of income.

4.8.3 Perception of street –kitchen vendors on the use of sanitizers

All the vendors who participated in the study said they used salt solution in washing cut cabbage pieces before used for their salad preparations. They also agreed that vinegar ($1:5^{v}/v$) is more effective for decontamination than the salt solution (an average concentration of 5 g/l). However, vendors preferred to use salt solution due to the high cost of vinegar. The adoption of vinegar as a decontaminant at the kitchen sites is therefore likely to be low.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Irrigation water quality

Most vegetables produced from urban farms are irrigated with water from streams, drains and shallow wells or dugouts which are presumably contaminated. To protect farmers and consumers from potential adverse health impact of wastewater use in agriculture, WHO has set a helminth guideline of ≤1 egg per litre and ≤1000 faecal coliforms per 100 ml levels in the irrigation water (WHO, 2006). Based on this guideline, the irrigation water quality assessed during the study period exceeded the guideline limits. A geometric mean of 1.05×10^4 per 100 ml and an average of 2.3 eggs / litre were obtained for thermotolerant coliforms and helminth eggs counts, respectively in the irrigation water. The irrigation water was therefore unacceptable for irrigating crops consumed raw. This confirms earlier results of a cross-sectional study conducted at the same site by Keraita *et al*. (2007a; 2007b) and Amoah *et al*. (2005).

Irrigation water used at the study site is drawn from shallow wells or dugouts, which receive a lot of contamination from surface runoff from surrounding farm environment. Poultry manure which is used as crop nutrient source is stored on the farm, and is easily blown by wind or carried into the irrigation water source. Moreover, farmers step in the shallow hand dug wells, to fetch water for irrigation. This practice disturbs the soil sediment and therefore causes redistribution of settled helminth eggs which eventually contaminate the irrigated cabbages. Another study by Keraita *et al*. (2008) on on-farm sedimentation ponds showed that, irrigation water sampled early in the morning before farmers used the ponds (hand dugout wells) for irrigation, and disturbed (unsettled) water during irrigation had an average difference of about 1.0 – 1.5 $\log_{10}$ thermotolerant coliforms per 100 ml and 4 helminth eggs per litre. Therefore, minimal disturbance of source of water would be effective in reducing thermotolerant coliforms and helminth eggs, contamination of irrigated cabbage. It would be beneficial if farmers' are trained in collection of irrigation water with minimal redistribution of sediments.

5.2 Reducing contamination on cabbage by cessation of irrigation

Many pathogens can survive long enough on crop surfaces to be transmitted to humans. Microbial contamination on cabbage can effectively be reduced by stopping irrigation for a reasonable number of days before harvesting. The results from this study revealed that an average daily reduction of 0.21 $\log_{10}$ unit thermotolerant coliforms and 0.1 helminth eggs per 100 g of cabbage are achievable in the dry season when wastewater irrigation is prevalent. However, these protections depend on climatic conditions (temperature, sunlight intensity), stoppage period or time, crop type, etc (WHO, 2006). Cessation of irrigation with wastewater before harvesting allows pathogens on vegetable to be killed if they are exposed to unfavourable climatic conditions such as high sunlight, temperature and dessication. Pathogen inactivation is much more rapid in hot sunny weather than under cool, cloudy, or rainy conditions (WHO, 2006).

The reduction in thermotolerant coliform numbers obtained for cabbage in this study (0.21 log units) was lower than the 0.5 – 2.0 $\log_{10}$ units reduction per day in the WHO (2006) guidelines (Table 4.7). This could be due to the fact that cabbage is a foliage crop and penetration of sunlight into the crop is substantially reduced. Foliage layers protect the surface of inner leaves therefore reducing the rate of die-off that result from exposure to sunlight. Higher reduction rate (3 $\log_{10}$ units per day) on lettuce was reported under very hot climatic conditions by Fattal *et al*. (2002). A similar study carried out by Keraita *et al*. (2007a) in Kumasi using lettuce as test crop also obtained a daily reduction of 0.65 $\log_{10}$ units for thermotolerant coliforms and 0.4 helminth eggs per 100 g lettuce. This could be due to the large surface area of lettuce exposed to the sunlight which enhances pathogen die –off. Farmers normally irrigate their cabbage till the day of harvesting to ensure freshness and reduce possible loss in their market value. Prolonged cessation of irrigation periods adversely affects the productivity and freshness of the lettuce (Keraita *et al*., 2007a; WHO, 2006).

The removal of outermost leaves of cabbage which are prone to direct contact with soil and irrigation water showed that, the intervention has good potential of reducing contamination levels on cabbage. An average of 1.4 $\log_{10}$ units of thermotolerant coliforms and 0.8 helminth eggs between outer and inner leaves were obtained. Furthermore, removal of three layers (i.e. 20-30%) reduced thermotolerant coliforms and helminth eggs contamination to levels that depict minimum or no detection of contamination therefore possess low health risk to consumers of cabbage. Minhas *et al*. (2006) also showed that removal of two outermost leaflets on cabbage reduces faecal coliform contamination drastically towards the centre of the cabbage. An average reduction of faecal coliforms from 1.8×10^2 to less than 2×10^0 MPN per 100 g was observed. Therefore, cabbage consumers can possibly reduce probable health risk due to pathogens if the two outermost leaves are discarded from whole cabbage before used for salad preparation and consumption.

5.3 Assessment of post-harvest handling practices in the market

Food markets have an essential function of providing consumers with safe and nutritious food (WHO, 2006) since markets are important components in the supply of vegetables to consumers and can influence contamination levels on vegetables. Contamination of vegetables could come from various sources in these markets. These include mode of displaying vegetables during marketing, other handling practices such as cutting cabbage prior to sales, and location of market.

This study, however, revealed that the modes of display of cabbage for sale in the market did not have much influence on the microbial recontamination. In the markets where this study was carried out, contaminated outer leaves are generally not removed before cabbage is displayed for sale. It was observed that wholesalers often purchase the cabbage from farmers and retail them in the market often displaying cabbage on the bare ground or on old sacks laid on the ground. The initial contamination of cabbage from the farm was not significantly increasing in the market outlets due to different modes of display. This observation confirms an earlier finding by Amoah *et al*. (2007) in Ghana in who reported that contamination of lettuce with pathogenic microorganisms did not significantly increase through post-harvest handling and marketing. On the contrary, Ensink *et al*. (2007) reported that significantly higher *E. coli* and helminth eggs were found on market vegetables in Pakistan than on vegetables harvested from the farm after 24 hrs. This significant increase from farm to market was also found by another study, in Portugal, by Vaz da Costa Vargas *et al*. (1996) who attributed the increase in faecal bacteria contamination to the use of contaminated refreshing water at the market.

Certain parts of vegetables, when in contact with wastewater and wastewater-irrigated soils, can prolong the survival of *E. coli* and in particular helminth eggs for longer periods (Feachem *et al.*, 1983). This present study showed that outer leaves removal reduced pathogen level significantly. Outermost leaves harbour most pathogens because of direct contact with sources of contamination. It was observed at the markets that, traders who normally remove one or two outer leaves of their cabbage display them on tables covered with clean sack. Informal interview with some of the retailers revealed that, the intention of removing outer leaves was to make the vegetable more attractive to customers rather than to reduce contamination.

This study has also showed that food preparation environment also influences recontamination of vegetables. Food prepared in hygienic environment can reduce consumer health risks. Cabbages cut in the market environment prior to selling had higher contamination levels than cabbages cut up in the laboratory. This was expected in view of unhygienic conditions in the market including lack of toilet facilities and tap water for washing hands after visiting places of convenience. A study by Nyanteng (1998) reported that only 32 % of markets in Accra have drainage system, 26 % have toilet facilities and 34 % are connected to pipe – borne water. The multiple use of the same knife in cutting cabbage and many other things without washing could result in cross contamination.

5.4 Market class environment influence on cabbage contamination

The markets selected for this study were classified into low, medium and high socioeconomic classes. This classification was based on the location of the market, handling practices at the market and the social class of people who patronize the market.

The low class market had the lowest level of contamination after outer leaves were removed. This is because those who patronize this market often do not purchase cabbage. Most of the sellers interviewed stated that, a bag of cabbages always took about two weeks on the shelf before it was completely sold out. Therefore, pathogen die-off is enhanced by the long stay of the cabbage on the shelf. Cabbage from Central market had the highest contamination levels among all the three markets because it is one of the main depots for wholesalers. It was observed, in this market, that the common mode of displaying cabbage was on old sacks partially covering the ground. Similar results that faecal coliforms population was high on cabbages sampled from Central market due to the handling practice was reported by Amoah *et al*. (2005). A fairly common handling practice at European market is washing of outer leaves of cabbage to get rid of soil particles. This practice was carried out to make the vegetables attractive to buyer.

5.5 Handling at consumer points

Washing under running tap has been prescribed for gross surface pathogen removal from vegetables. The effect of washing cabbage leaves under running tap for two minutes before cutting into pieces showed that adequate washing is an effective means of reducing contamination levels on cabbage. This is because removal of helminths eggs required a physical force which can be provided by the running tap water. As the running water flushes the leaves, pathogens are dislodged and washed away. This also ensures the elimination of contamination likely to occur when washing is done in a bowl of water. Significant reduction levels of contamination were obtained in similar study by Amoah *et al*. (2007a) using lettuce. Other studies have reported that approximately 1 to 2 log reductions (depending on nature of surface of leaves) can be achieved when vegetables are washed vigorously under running tap water (Beuchat, 1998; Lang *et al.*, 2004). However, Rosas and Baez (1984) found that rinsing of vegetables with tap water in a bowl reduced indicator coliform population levels but not to the safe level recommended by the ICMSF (1974). In Ghana, however, this method has a limited application potential because of absence of running taps in poor households *(*Amoah *et al.*, 2007a).

There is a general practice in the households to wash vegetables with or without sanitizer before consumption. A survey conducted by Amoah *et al*. (2007a) in Ghana revealed that various sanitizers including vinegar, salt and chlorine tablets are used in washing vegetables at the kitchen before consumption. According to Parish *et al*. (2003), the efficacy of the method used to reduce microbial populations usually depends on the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces, exposure time and concentration of sanitizer, pH, and temperature. Results from this study show that vinegar is more effective in reducing contamination on cabbage than salt solution (Table 4.6). This is in agreement with observation made by Amoah *et al*. (2007a). This could be due to the concentration of the salt solution used in washing the cabbage pieces since it was prepared to taste of the food vendor. Informal interview revealed that, this is the normal practice. This low concentration of the sanitizer might account for the low reducing power of the salt solution as compared to vinegar.

Recent study shows that higher salt concentration of 23 and 35 mg/l had a considerable deteriorating effect on lettuce leaves (Amoah *et al*., 2007a). Vinegar is more effective in de-contaminating vegetables but more expensive than common salt. Because vinegar is not easily affordable, the potential of adoption by street kitchen vendors is low.

5.6 Cumulative effectiveness of the non-treatment interventions in reducing contamination of wastewater- irrigated cabbage

Disability Adjusted Life Years (DALY) is the measure of the health of a population or burden of disease due to a specific disease or risk factors. Based on the exposure scenarios of vegetable consumption and relevant epidemiological evidence, it has been shown that, in order to achieve $\leq 10^{-6}$ Disability Adjusted Life Years (DALY) per person per year, a total pathogen reduction of 6 log units for consumption of leafy crops (e.g. lettuce) and 7 log units for consumption of root crops (e.g. Onions) is required. In the new WHO (2006) guidelines, a number of measures (interventions) have been proposed with each intervention having its associated log reduction or range of reduction (WHO, 2006). This study revealed that each non-treatment intervention imposed at the different stages (i.e. farm, market, and kitchen) along the producer-consumer chain showed varied effectiveness in reducing contamination levels (Table 4.6). For example, cessation of irrigation before harvesting on-farm reduced thermotolerant coliforms by 0.21 log units per day and 0.6 helminth eggs per 100 g wet weight cabbage. Good handling practices at the market such as removal of outermost leaves and display of cabbage on tables covered with clean sacks, led to significant reduction in the contamination levels. These interventions led to further reduction of 0.97 log units of thermotolerant coliforms and

0.2 helminth eggs at the market. However, the WHO Guidelines (2006) do not include any intervention for market and helminth eggs reduction associated with each intervention, and therefore makes comparison with established results difficult. Washing of cabbage at the kitchen with a sanitizer was able to reduce contamination levels considerably.

Generally, the cumulative reductions obtained for the range of interventions including vinegar was 3.91 $\log_{10}$ units for thermotolerant coliforms and 1.4 helminth eggs per 100 g wet weight, while with salt solution yielded 3.07 $\log_{10}$ units thermotolerant coliforms and 1.5 helminth eggs. These cumulative reductions obtained were lower than the 6 – 7 log reductions proposed in the guideline. This is because all the combination options proposed in the WHO (2006) guideline for the performance target of 6 -7 log reduction included wastewater treatment (between 3 – 4 log reductions) in combination with other interventions. For instance, option A in the guideline showed that pathogen reduction is achieved by combination of (a) wastewater treatment, which provides a 4 log unit pathogen reduction, (b) pathogen die – off between the last irrigation and consumption (a 2 log unit reduction) and (c) washing the vegetable with water prior to consumption (a 1 log unit reduction). This option provides a 7 log unit pathogen reduction which is suitable for root crops eaten raw. The difference however is that this study focused on combination of non-treatment interventions on the vegetable and has not considered pathogen reduction from the wastewater treatment. The WHO set target of 6 – 7 pathogen reduction can easily be achieved through adequate cooking of the vegetable (WHO, 2006).

5.7 Types of helminth eggs identified

The number of different types of helminth eggs isolated from the irrigation water exceeded the WHO (2006) guideline limits of ≤ 1 helminth egg per 100 ml for unrestricted irrigation water. At all the cabbage sampling points, *Ascaris lumbricoides* was the predominant helminth eggs type isolated. This is supported by the results obtained by Amoah *et al*. (2005) who recorded that *Ascaris lumbricoides* formed the greater proportion of helminth eggs isolated on lettuce leaves from Kumasi. *Ascaris lumbricoides* can remain viable for several months or years in soil, although often less than 2 months on crops compared to other helminth eggs such as *Schistosoma species* which are unlikely to survive for more than couple of days (WHO, 2006). Feachem *et al*. (1983) showed that *Ascaris lumbricoides* can survival in different environment such as faeces, sludge, freshwater, sewage, and in soils for many months and usually on crop for < 60 days, while the other helminth eggs can survive in these environment between <10 - 30 days. The relatively low numbers of the other helminth eggs such as *Schistosoma species*, *Trichuris trichiura* isolated could be due to their low survival period in the environment.

5.8 CONCLUSION

This study showed that the irrigation water, (which is mainly from shallow hand dug wells) used in vegetable farming, is highly contaminated with thermotolerant coliforms and helminth eggs, and the contamination levels are above the safe recommended level. Therefore cabbages produced from these irrigation water sources are also contaminated. The levels of contamination need to be reduced to eliminate potential health risk associated with consumption of wastewater irrigated cabbage. The study carried out showed that, cessation of irrigation four (4) days prior to harvesting significantly reduce thermotolerant coliforms and helminth eggs counts. Removal of three outer leaves (about 30 % of the whole cabbage weight) resulted elimination of thermotolerant and helminth eggs population, but resulted in reduction of market value of the cabbage.

The location and environmental quality of the different markets also played a vital role in the recontamination of cabbage. Assessment of post-harvest handling practices at the market indicated that different modes of displaying cabbage for sale in the markets contributed to increased contamination levels of cabbage. However, removal of one outer leave can be a simple non-treatment intervention at the market for reducing contamination levels.

The "multiple barrier approach" where barriers (non – treatment interventions) were placed along the production – consumption pathway to have aggregate effect in reducing health risks in line with the WHO (2006) guidelines, is feasible in the Ghanaian context.

Significant cumulative reductions were obtained for both thermotolerant coliforms and helminth eggs when a 4-day cessation, removal of outermost leaves and displaying cabbage on clean sack on table and different sanitizers were tested in combinations. In all, the study indicted that, non-treatment interventions proposed in the WHO (2006) guideline are effective under actual field conditions in reducing contamination levels on wastewater - irrigated cabbage in Ghana.

5.9 RECOMMENDATIONS

Ensuring food safety to protect public health and promote economic development remains a significant challenge in developing countries where wastewater is used in vegetable farming. Guideline information on the best ways to maximize wastewater reuse and reduce potential health risk in the increasing waning water resources while maintaining food safety is needed.

It is recommended that:

1. Vegetable farmers using wastewater should be educated on the benefits of the various interventions and encouraged to adopt them to reduce on -farm contamination.
2. Vegetable sellers at the market should be educated on improved post-harvest handling practices and encouraged to adopt them to reduce contamination.
3. Street kitchen vendors should be educated and encouraged to use improved washing methods during food preparation.
4. Education of all stakeholders (farmers, sellers and street kitchen vendors) along the "multiple barrier approach" pathway about the essential role of each of them to reduce recontamination.
5. Further studies should be carried out on Quantitative Microbial Risk Assessment (QMRA) to quantify the actual risk of disease associated with consumption of wastewater - irrigated vegetables and calculate Disability Adjusted Life Year (DALY), values appropriate for Ghanaian communities.

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APPENDICES

Appendix 1: ANOVA FOR CESSATION OF IRRIGATION BEFORE HARVESTING

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotoleran coliforms	Between Group	22.681	3	7.560	21.688	.000
	Within Groups	23.705	68	.349		
	Total	46.386	71			
Log helminth eggs	Between Group	.135	3	.045	1.511	.219
	Within Groups	2.028	68	.030		
	Total	2.163	71			

Appendix 1A: Multiple Comparisons Analysis for the different cessation period of irrigation

Dependent Variable: Log Thermotolerant coliforms

LSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
.00	4.00	.99620*	.19681	.000	.6035	1.3889
	8.00	1.30343*	.19681	.000	.9107	1.6962
	12.00	1.43137*	.19681	.000	1.0386	1.8241
4.00	.00	-.99620*	.19681	.000	-1.3889	-.6035
	8.00	.30723	.19681	.123	-.0855	.7000
	12.00	.43516*	.19681	.030	.0424	.8279
8.00	.00	-1.30343*	.19681	.000	-1.6962	-.9107
	4.00	-.30723	.19681	.123	-.7000	.0855
	12.00	.12793	.19681	.518	-.2648	.5207
12.00	.00	-1.43137*	.19681	.000	-1.8241	-1.0386
	4.00	-.43516*	.19681	.030	-.8279	-.0424
	8.00	-.12793	.19681	.518	-.5207	.2648

*. The mean difference is significant at the .05 level.

Appendix 1B : Contrast Tests between control and other treatments

		Contras	Value of Contrast	Std. Error	t	df	Sig. (2-tailed
Log thermotole coliforms	Assume equal vari	1	2.4449[a]	.32712	7.474	42	.000
	Does not assume e	1	2.4449[a]	.35795	6.830	35.000	.000
Log helminth e	Assume equal vari	1	.0431[a]	.01956	2.202	42	.033
	Does not assume e	1	.0431[a]	.01803	2.389	35.000	.022

a. The sum of the contrast coefficients is not zero.

Appendix 2: Anova for percentage removal of outermost leaves on cabbage from farm

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotoleran coliforms	Between Group	205.148	4	51.287	61.758	.000
	Within Groups	33.218	40	.830		
	Total	238.366	44			
Log helminth eggs	Between Group	.917	4	.229	21.315	.000
	Within Groups	.430	40	.011		
	Total	1.347	44			

Appendix 3 : ANOVA FOR DIFFERENT MODES OF DISPLAYING CABBAGE DURING MARKETING

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant Coliforms	Between Groups	2.506	2	1.253	.893	.422
	Within Groups	33.668	24	1.403		
	Total	36.174	26			
Log Helminths eggs	Between Groups	.042	2	.021	.560	.578
	Within Groups	.904	24	.038		
	Total	.947	26			

Appendix 4: ANOVA FOR OUTERMOST LEAVES REMOVAL AT MARKET

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotoleran coliforms	Between Group	7.292	1	7.292	17.962	.000
	Within Groups	8.931	22	.406		
	Total	16.222	23			
Log Helminth eggs	Between Group	.202	1	.202	2.643	.118
	Within Groups	1.678	22	.076		
	Total	1.880	23			

Appendix 5: ANOVA FOR CUTTING CABBAGE PRIOR TO SELLING AT MARKET

		Sum of Squares	df	Mean Square	F	Sig.
Log Thermotolerant coliforms	Between Groups	34.329	1	34.329	14.510	.001
	Within Groups	52.051	22	2.366		
	Total	86.379	23			
Loh Helminth eggs	Between Groups	.109	1	.109	2.075	.164
	Within Groups	1.157	22	.053		
	Total	1.266	23			

Appendix 6: ANOVA FOR WASHING EFFECT ON CABBAGE

		Sum of Squares	df	Mean Square	F	Sig.
Log thermotoleran coliforms	Between Groups	52.941	1	52.941	188.829	.000
	Within Groups	6.168	22	.280		
	Total	59.109	23			
Log helminth eggs	Between Groups	.516	1	.516	38.350	.000
	Within Groups	.296	22	.013		
	Total	.811	23			

Appendix 7 ANOVA FOR VINEGAR AS SANITIZER

		Sum of Squares	df	Mean Square	F	Sig.
log thermotoleran coliforms	Between Group	47.930	1	47.930	26.125	.000
	Within Groups	73.386	40	1.835		
	Total	121.316	41			
log helminth eggs	Between Group	.000	1	.000	.013	.909
	Within Groups	.716	40	.018		
	Total	.716	41			

Appendix 7A ANOVA FOR SALT SOLUTION AS SANITIZER

		Sum of Squares	df	Mean Square	F	Sig.
log thermotoleran coliforms	Between Group	16.777	1	16.777	14.862	.000
	Within Groups	45.154	40	1.129		
	Total	61.931	41			
log helminth eggs	Between Group	.137	1	.137	5.988	.019
	Within Groups	.916	40	.023		
	Total	1.053	41			

Appendix 8: Anova for cessation during cummulative reduction study

		Sum of Squares	df	Mean Square	F	Sig.
log thermotolera coliforms	Between Grou	7.261	1	7.261	43.992	.000
	Within Groups	6.602	40	.165		
	Total	13.862	41			
log helminth egg	Between Grou	.174	1	.174	4.207	.047
	Within Groups	1.650	40	.041		
	Total	1.824	41			

Appendix 8A: Anova for outermost leaf removal in the market during the cummulative reduction study

		Sum of Squares	df	Mean Square	F	Sig.
log thermotolerant coliforms	Between Groups	9.885	1	9.885	49.885	.000
	Within Groups	7.926	40	.198		
	Total	17.811	41			
log helminth eggs	Between Groups	.006	1	.006	.116	.735
	Within Groups	2.052	40	.051		
	Total	2.058	41			

Appendix 9: Characteristics features of helminth eggs for identification (WHO, 1994)

Ascaris lumbricoides: Morphologically, the eggs produced are of two types namely; Fertile and infertile eggs. The fertile eggs are oval to round, 55µm to 75µm long by 35µm to 50µm wide with a thick lumpy outer shell (mammillated, uterine, or proteinatious layer) that is contributed by the uterine wall. There is one cell inside the egg which is separated from the shell at both ends. When the eggs are passed out in faeces they are golden yellow to brown in colour. The egg has a conspicuous mamillations on its outer surface. Sometimes, the normal fertile eggs lack the mamillated layer and are referred to as "decorticated" eggs. The infertile eggs are elongated and much larger in size measuring 85-95 by 43 to 47µm wide. The eggs have thin shells and a grossly irregular mamillated layer. The content of the egg is usually granular and lacks any organization.

Trichuris trichura: These measure 50 to 55 by 22 to 24µm. They are elongated and lemon shaped with a brown, smooth shell, bipolar prominences (plugs) at each end. At the time the egg is laid, it contains a single-cell ovum.

Hookworm (eggs of *Necata americanus*): These eggs are characteristically barrel-shaped with a thin, hyaline shell; they measure 65 to 75by 36 to 40µm. The egg is colourless with grayish cells. They are usually in the 4 to 8 cell stage in faeces or in a more advanced stage of cleavage in samples that have been kept at room temperature for even a few hours.

Schistosoma mansoni: These are about 114 to 175µm by 45 to 70µm. They have a thin, elongated, transparent shell and a prominent lateral spine near the posterior end. The anterior end is tapered and slightly curved. The colour is yellowish brown or yellow. It is embryonated and contains a miracidium.

Schistosoma haematobium: These have a size of 112 to 170µm by 50 to 70µm. It is elongated with rounded anterior end and a terminal spine at the posterior end. It is embyonated and contains a matured miracidium.

Stongyloides stercoralis: The first-stage rhabditoid larvae measures 180 to 380µm by 14 to 20µm. The larvae have a short buccal capsule, an attenuated tail and a prominent genital primordium.

Taenia spp: The eggs are all identical, i.e. 31 to 43µm in diameter, with a thick, prismatic-appearing shell wall, and contain a 6-hooked embryo, the oncoshpere. Occasionally a thin, hyaline primary embryonic membrane may be retained around the eggs.

Clonorchis sinensis: The eggs are 27 to 35µm by 12 to 19µm. they have a seated operculum and usually a small protuberance at the opposite end. The shell may have minute adherent debris. Eggs from faecal origin contain a miracidium.