**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI**

COLLEGE OF SCIENCE

**FACULTY OF BIOSCIENCES**

**DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY**

# HELMINTH CONTAMINATION OF LETTUCE AND ASSOCIATED RISK FACTORS AT PRODUCTION SITES, MARKETS, AND STREET FOOD VENDOR SITES IN URBAN AND PERI-URBAN KUMASI.

**A THESIS PRESENTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY, COLLEGE OF SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (ENVIRONMENTAL SCIENCE)**

**BY**

**LINDA AURELIA ANDOH**

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## DECLARATION

I hereby declare that this thesis presented to the Department of Theoretical and Applied Biology in partial fulfillment for the award of MSc. Degree, is a true account of my own work except for the references that have been duly acknowledged.

Signature…………………………… Date………………………….

NAME: ANDOH LINDA AURELIA

(STUDENT)

Signature…………………………….. Date……………………………

NAME: PROF. R. C. ABAIDOO

(SUPERVISOR)

## DEDICATION

To my father, Nana Kwantwi Berimah II and Mother, Esther Darko-Kumi

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**TABLE OF CONTENT**

**LIST OF PLATES** ............................................................................ **Error! Bookmark not defined.**

**ABSTRACT**

In the urban cities of many developing countries demand for fresh vegetables has necessitated the use of wastewater for irrigation. Microbial load on these vegetables at both the production and the distribution points is reportedly high. The study assessed the helminth parasites contamination on lettuce and associated risk factors at farm, market and the fast food street vendor points in Kumasi. Three farms, three market sites where lettuce from the selected farms is sold and thirteen street food vendors who purchase their lettuce from these markets were studied. Samples of lettuce, irrigation water and refreshing water (water used in keeping lettuce fresh throughout the day) were collected from these sites and analyzed for helminth eggs using standard methodology (Schwartzbrod, 1998). Helminth eggs on lettuce leaves, irrigation water and refreshing water on the farms and markets were mostly *Ascaris lumbricoides* with others being *Shistosoma, Hookworm, Trichuris trichura, Taenia, Clonorchis* and Strongyloides larvae. Helminth eggs on lettuce ranged between 4 and 14 eggs/100g wet weight and 3 and 25 eggs/l in irrigation water on the farms, and between 2 and 7 eggs/100g wet weight and 4 to 15 eggs/l in refreshing water on the markets. Helminth egg counts on lettuce leaves on two farms were 25-36% more compared to the farms irrigation water but one farm had 25% more in irrigation water compared to the lettuce leaves. Helminthes eggs on two farms lettuce were 33.3% and 42.9% higher compared to the corresponding markets samples but 10.5% higher one market compared to its farm source. Helminth eggs in street food lettuce samples analysed from the selected areas were only *Ascaris* and *Shistosoma* eggs ranging between 0 to 2eggs/100g wet weight. Helminth egg numbers for both farm and market samples exceeded the recommended level of <1egg $l^{-1}$ (WHO, 1989). Education on farm practices, post harvest handling and washing methods at both market and street food vendor sites and improved hygienic practices at consumer level will help reduce their numbers and minimize the risk.

# CHAPTER ONE

## 1.0 INTRODUCTION

## 1.1 BACKGROUND

The use of wastewater for irrigation in agriculture has been practiced for many years in arid zones of industrialized as well as developing countries. The persistence in the use of this water resource has been attributed to several factors, including the decreasing availability of water resources for irrigation as a result of the increasing demand for potable water in urban and peri-urban communities, the high cost of artificial fertilizers and the realization that nutrients in wastewater can increase crop production and the social acceptance of the practice (Mara and Cairncross, 1991).

For the Ghanaian economy, agriculture contributes 36.6% of the gross domestic product (GDP) and employs 60% of the labour force. The average annual *per capita* income of those employed in agriculture is estimated at US$390 ( CIA World Fact Book, 2005).Irrigated agriculture is therefore important in peri-urban communities producing about 90 % of vegetables consumed in the cities and also providing a major source of income for the households (Drechsel *et al.*, 2001). The growing demand for fresh and perishable agricultural produce in the major cities is driving the development of peri-urban agriculture. This demand is not seasonal as vegetable farming is done all year round. The vegetables commonly grown include cabbage (*Brassica oleracea capitata*), spring onions (*Allium fistulosum*), carrots (*Daucus carota ssp.sativus*), tomato (*Lycopersicon esculentum*), onion (*Allium cepa*), Shallots (*Allium escalonicum*), egg plant (*Solanum melongena*) local spinach (*Amaranthus* spp), cucumber (*Cucumis sativa*) and lettuce (*Lactuca sativa*) which is eaten raw (Sanduri, 2004).

It has been reported that vegetables can be contaminated by microrganisms at both the production and the distribution points (Drechsel *et al.,* 2000) and this has been attributed to the irrigation water, sources of crop nutrients (organic manure) and handling and storing of produce at points of sale.

Wastewater reuse may therefore lead to public health risks of transmission of enteric diseases following the consumption of raw vegetables. These include waterborne and foodborne transmission of helminths, particularly nematodes and endemic parasitic diseases to which children are most vulnerable (HCWC, 1994; Shuval *et al.,* 1986). Pathogenic bacteria such as *Escherichia coli* and *Salmonella* have also been implicated (Sonou, 2001).

## 1.2 PROBLEM STATEMENT

The use of polluted and or marginal quality water for irrigating vegetables has been done for ages. These irrigation water sources are known to be contaminated and in most cases exceed the WHO guidelines significantly (Keraita et al., 2003b)

Some researchers have shown that in the production-consumer pathway of lettuce, markets form an important aspect. Sellers mainly women buy them on-farm from field beds that have been ordered in advance and transport them to these markets where they are sold in bulk or retailed to consumers (Danso and Drechsel, 2003). According to Drechsel *et al* (2000), as consumers demand fresh and clean but not safe vegetables, refreshing and cleaning with water often of as bad quality as irrigation water is thus the normal practice in markets. It has been documented by Drechsel *et al* (2000) that it is difficult to find on markets, any irrigated vegetables (lettuce, spring onions, and cabbage) which are not contaminated with faecal coliforms and helminths eggs. It is therefore hypothesised that the contamination of lettuce sold in markets is significant and higher than its contamination on arrival from the farm.

The rapid growth of the urban population (2.7%) and the increasing number of working people have caused changes in the eating habits of Ghanaians (Ghana Statistical Service, 2002). Consequently, fewer people, especially in the urban areas, are eating home-cooked meals as much food is purchased from vendors of the fast-food industry which is also growing rapidly. A mini-census and a survey by the National Resources Institute of 334 street vendors in Accra, Ghana indicated that the street food sector employs over 60,000 people and has an estimated annual turnover of over US$100 million (FAO, 2005). Despite the contributions of street foods to food security and local economies, several outbreaks of foodborne diseases have been traced to the consumption of contaminated street foods which in most cases come with lettuce as part of the salads. Numerous studies have been documented on the potential contamination of street foods by pathogenic microorganisms but the source and contribution of helminth parasites to this contamination is not very clear.

## 1.3 RESEARCH QUESTIONS:

1. What is the quality of the irrigation water used in lettuce production in urban and peri-urban Kumasi?
2. What is the quality of the lettuce sold in the market compared to those on farm in terms of Helminth numbers?
3. What is the quality of the lettuce sold at the street food vendor sites compared to those at the market and the farm?
4. What are the key risk factors associated with human health, including those related to hygienic conditions and practices of producers, sellers and consumers of waste water irrigated lettuce?

## 1.4 OBJECTIVES OF STUDY

### 1.4.1 General Objectives

This study will focus on the contamination of lettuce (which is normally consumed raw and perhaps the most perishable of all the vegetables cultivated and sold in most cities in Ghana.) and associated risk factors at production sites, in markets and street food vendors in urban and peri-urban Kumasi.

### 1.4.2 Specific Objectives

1. To assess the microbial quality of irrigation water in relation to helminths eggs contamination.
2. To assess contamination levels of helminth parasites in lettuce at selected farm sites, markets and street vendors in urban /peri-urban Kumasi
3. To identify risk factors associated with faecal contamination of lettuce at farm sites, markets and street food vendors.
4. To develop interventions to reduce the risks of faecal contamination of lettuce.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

## 2.1 TERMS AND DEFINITIONS

The following paragraphs give working definitions of different terms used in this work.

In many developing urban areas, wastewater is generally a mixture of the three different categories and its use is mainly informal. The uncontrolled and varied nature of sources of wastewater used for irrigation makes it difficult to define, monitor and control the practice. Van der Hoek, (2004) classifies wastewater use into:

- Direct Use- the planned use of raw or treated wastewater where control exists over the conveyance of the wastewater from the point of collection or discharge from treatment works to a controlled area where it is used for irrigation. This is the situation pertaining in most developed nations where physical and institutional infrastructure is well established to monitor and control the quality of the water and the area where it is used for irrigation.
- Indirect Use- the situation found in many developing countries where much municipal and industrial wastewater is discharged without treatment, monitoring or control into the water courses draining an urban area. The resulting water quality varies.

Cornish *el al.,* (1999) classified wastewater as:

a. Raw/Untreated Wastewater- liquid discharged from homes or commercial premises to individual systems or municipal sewers. It is a mixture of domestic sewerage- dirty water, human excreta and municipal waste water. It may not contain substantial quantities of industrial effluent.

b. Treated /partially treated wastewater- wastewater that has been treated by a natural or artificial purification process to improve its physical, chemical or bacteriological quality before discharge into a surface water body. It may still pose a threat to receiving environments.

c. Industrial Effluent - wastewater from industrial processes and containing high levels of heavy metals or other chemicals or organic constituents. Industrial effluent does not normally contain high levels of microbial pollutants.

Wastewater is also categorized according to its origin. The categories include:

1. **Grey water**: composed of domestic water without urine and faeces
2. **Black water**: composed of domestic water that is mixed with faeces and urine.
3. **Industrial wastewater**: composed of water from industrial processes which may contain varying concentration of heavy metals.

Marginal Quality - this term refers to water which is 'marginal' for use in agriculture. Abbott and Hasnip (1997) define it as "water which might pose a threat to sustainable agriculture and or human health by virtue of its quality but which can be used safely for irrigation provided certain precautions are taken". It describes water, which has been polluted as a consequence of mixing with wastewater or agricultural drainage.

## 2.2 IRRIGATION IN KUMASI

Kumasi has a semi humid, tropical climate with a total average rainfall of 1340mm. approximately 90% of the annual total falls between March and October. Natural drainage runs from north to south. The principal streams in the area are the Daban, Subin, Aboabo, Sisa and Wiwi. These converge into the Sisa which flows into the Oda River about 9 km south of Kumasi. Small scale irrigated agriculture is done in the dry season in many villages within 40km radius of Kumasi. In other areas, farmers draw water either from ephemeral streams which flow from a series of pools in the dry season or from shallow hand-dug wells. Farmers' postulate that water drawn from shallow wells are is better in quality than that from the rivers.

## 2.3 SOURCES OF CONTAMINATION OF VEGETABLES

Parasites contaminate crops through various routes taken during pre-harvest, harvest and post-harvest for example, via water contaminated by faeces that are used for irrigation or spraying of crops, by poor personal hygiene practices among handlers of crops, by contact with contaminated soil or by contact with faeces of wild animals. Thus, pathogens from the human and animal reservoir as well as other pathogens from the environment can be found on crops at the time of consumption.

## 2.4 Sources of pathogenic microorganisms on fresh fruits and vegetables

*Adapted from Beauchat and Ryu (1997)*

| *Pre-harvest* | *Post harvest* | *Sorting, packing and further processing equipment* |
|---|---|---|
| • Feces<br>• Soil<br>• Irrigation water<br>• Water used to apply<br>• fungicides,<br>• insecticides<br>• Green or inadequately composted manure<br>• Air (dust)<br>• Wild and domestic animals (including fowl and reptiles)<br>• Insects<br>Human handling | • Feces<br>• Human handling (workers, consumers)<br>• Harvesting equipment<br>• Transport containers (field to packing shed)<br>• Wild and domestic animals (including fowl and reptiles)<br>• Insects<br>• Air (dust)<br>• Wash and rinse water | • Ice<br>• Transport vehicles<br>• Improper storage (temperature, physical environment)<br>• Improper packaging (including new packaging technologies)<br>• Cross-contamination (order foods in storage, preparation, and display areas)<br>• Improper display temperature<br>• Improper handling after wholesale or retail purchase |

### 2.4.1 Pre-harvest

**Soil**

In the preparation of soil for planting, farmers mostly use uncomposted organic manures to fertilize the soil. These manures contain several microorganisms (Behrsing *et al*, 2000). Soil is known to be a rich source of a variety of microorganisms and the non-pathogenic flora is important for the mineralization of plants and animals after their death in the environment.

Pathogenic organisms from the human/ animal reservoir can be found in the soil due to irrigation and fertilization with manure and sludge or due to droppings of animals in the farming area. Tissue degrading properties of this flora contaminating vegetables may cause damage during transport and storage of products thereby exposing them to further microbial attack.

**Irrigation Water**

Water is mainly used for irrigation of plants and its quality varies depending on whether it is surface water or potable water. A recent study of two sites in the Accra Metropolitan Area (Sonou M. *et al*, 2001) revealed that wastewater was the most frequently used water for irrigation purposes. As much as 60% of the farmers interviewed at Dzowulu Power Pool Station (67.7%) and at Castle Parks and Gardens (32.3%) confirmed the use of this type of water. Less than a quarter (23.3%) use pipe-borne water while approximately 17% use piped water stored in a ground reservoir.

Irrigation water may be a source of contaminating microorganisms. Surface water from streams and lakes may be contaminated with pathogenic protozoa, bacteria and viruses. The occurrence of *L. monocytogenes*, *Salmonella* and viruses has been reported (Castillo Martín *et al.*, 1994; Nguyen and Carlin, 1994 and 2000).Water from sewage plants can be used for irrigation purposes but without further hygienic treatment, it may represent a risk for contaminating the crop.

Giardia cysts have been detected on fresh vegetables like carrots, mint, radish and potatoes irrigated with wastewater (Lane and Lloyd, 2002, Amahmid *et al*, 1999). Of waters used to irrigate vegetables in the USA and several American countries, 60% tested positive for Giardia cysts and 36% tested positive for cryptosporidium oocysts (Thurston-Enriquez *et al*, 2002).

**Organic Fertilizers**

In enhancing production of the vegetables there is the need for fertilizers. Inorganic fertilizers are however very expensive hence, the use of organic manure which is inexpensive. Sewerage, manure, slurry, sludge and compost of human and animal origin are commonly used as organic fertilizer for vegetable production particularly in organic production systems. Of all the organic manures however, poultry manure seems to be the cheapest and the most preferred.

In Kumasi, the use of poultry manure is very common due to its high availability and low price (US $0.1 per sack). Only a few farmers use mineral fertiliser in addition to this (mostly for cabbage production). In urban Kumasi, many more vegetable farmers use mineral fertilisers (US $14 per 50kg NPK) but combine it with poultry manure when possible(Danso et al., 2003).

Usage however of the poultry manure may have its adverse effects just as most inexpensive alternatives do. The faecal origin of these fertilizers, however, indicates a potential risk of contamination by viruses, bacteria and parasites pathogenic to humans.

In Belgium and Finland, Listeria monocytes was found in 6.7 to 20% of the samples analysed (Husu, 1990; Van Renterghem et al, 1991) and in sewerage sludge (stauch, 1991).

Poultry manure, which represents 75% of the organic fertiliser used, generally contains faecal coliforms ($1.30x10^6$/g) and enterococci ($3.4x10^6$/g) (Westcot, 1997). This is evident even in situations where pipe-borne water was used for irrigation signifying that the contamination was from the poultry manure. Vegetables cultivated with manure are highly infected by bacteria, indicating contamination from a faecal source (Sonou et al., 1999).

The concentration of microorganisms in animal manures is of major importance because this determines the quantity of bacteria which reaches soils and groundwater.

Helminth and protozoan parasites enter the environment in faeces from the intestinal tract of a wide range of domestic, wild and companion animals used as manure for production.

In general, increasing the delay between the application of organic fertilizers and harvest could reduce the occurrence of food borne pathogens on vegetables.

### 2.4.2 Harvest

Vegetables can become contaminated with pathogenic microorganisms during harvesting through faecal material, human handling, harvesting equipment, transport containers, wild and domestic animals, air, transport vehicles, ice or water (Beuchat, 1995).

In an investigation of several food borne illnesses associated with fresh produce (NACMCF, 1999a), agricultural workers were in many cases the likely source of the pathogen. Lack of suitable sanitary hand-washing facilities in the production area can potentially create a hygienic problem.

Clean, well-designed and maintained equipment is less likely to cause damage to fresh produce and to introduce spoilage and pathogenic microorganisms (Brackett, 1992). Dirty storage facilities and the presence of rodents, birds and insects may increase the risk of contamination with food borne pathogens (FDA, 1998). Finally, harvesting at the appropriate time and keeping the harvested product under controlled environmental conditions will help retard growth of post-harvest spoilage (Brackett, 1992) and pathogenic microorganisms.

### 2.4.3 Post Harvest Sources

The fecal-oral route of transmission of pathogens broadens to include workers handling vegetables from the point of removal from the plant through all stages of handling, including preparation at the retail and food service levels and in the home. Traditionally recognized post harvest control points for access of pathogens to whole or cut produce include transport containers and vehicles.

Post harvest treatment of vegetables includes handling, storage, transportation, and sorting, packing, cutting, cleaning and further processing equipment. Conditions arise during these practices which lead to cross contamination of the produce from other agricultural materials or from the workers. Environmental conditions and transportation time also influences the hygienic quality of the produce prior to processing or consumption. Poor handling damages fresh produce, rendering them susceptible to the growth/survival of spoilage and pathogenic microorganisms.

Another main source of microbiological contamination at the market level is poor handling and storing practices of vegetables by market women. Vegetable sellers wash the vegetables in water before selling them. Observation of the storage conditions has, however, revealed that the vegetables are generally exposed and are frequently visited by houseflies and other insects including cockroaches. The common micro-organisms isolated from vegetable samples include *E. coli*, *Pseudomonas, Enterobacter cloacae, Salmonella arizonae* (Sonou, 2001). Other organisms (helminthes and protozoan) identified on vegetables collected from the field and market includes free-living soil nematodes, flagellates and Balantidium coli.

## 2.5 WASHING AND DECONTAMINATION

Washing of vegetables at harvest removes much of the adhering soil and dirt. However, it could also be a source of microbial contamination. Even where washing is applied, effective washing and decontamination of ready-to eat vegetables is difficult. Refreshing and cleaning vegetables with water often as bad quality as irrigation water is thus normal practice in most markets (Dreschel *et al.,* 2000).

Chlorine is the major compound used for disinfection of fresh produce. During sprouting of seeds chlorine can be used in the water to prevent growth of contaminating microorganisms. Though the effect of disinfectants on contaminants depends on many factors including the concentration used, treatment time, temperature, pH and sensitivity of the target organism(s), the most effective form is hypochlorous acid (HOCl) (Simons and Sanguansri, 1997) and chlorine concentration of 100 ppm is frequently used. However, the use of chlorine does not ensure elimination or even an efficient reduction in pathogen levels. Other substances many be used including organic acids, chlorine dioxide, hydrogen peroxide and ozone (Beuchat, 1998). Organic acids alone, or in combination with chlorine, have been shown in experimental designs to effectively reduce the number of pathogens for example, *Yersinia enterocolitica* and *Listeria monocytogenes* in parsley (Karapinar and Gonul, 1992; Zhang and Farber, 1996).

Prevention of contamination at all points of the food chain is preferred over the application of disinfectants.

## 2.6 USE OF UNTREATED AND PARTIALLY TREATED WASTEWATER FOR IRRIGATION

A number of existing studies have documented the prevalence of high intestinal nematode infections found among farmers and their family members who are in direct contact with wastewater (Krishnamoorti et al, 1973; Shuval et al., 1986; Habbari et al., 1999; Blumenthal and Peasey). Many of these studies are descriptive and have not tried to quantify relative or attributable risk of infection as a result of the use of untreated wastewater for irrigation. An even smaller number of studies have tried to link exposure to a known intestinal nematode concentration and the risk of human nematode infections (Blumenthal et al, 2000).

The different studies on the use of untreated wastewater in agriculture all show similar outcomes with a clearly increased risk of hookworm and *Ascaris* infections for adult wastewater farmers. A study of Indian farmers using untreated wastewater showed a more than three-fold increased risk of *Ascaris* infections, and a more than two-fold increased risk of hookworm infections as compared to “regular” farmers (Krishnamoorthi et al, 1973). In Haroonabad, a small town in Pakistan, hookworm prevalence in farmers using wastewater with a nematode egg load of 100 eggs/litre was very high, with 80% of the farmers infected. The study showed a more than four-fold increased risk for wastewater farmers as compared to farmers using regular irrigation water. *Ascaris* prevalence was found to be low in both wastewater farmers as well as regular farmers and no increased risk was found between both groups. A much lower hookworm prevalence was found in children and no excess risk was found for both *Ascaris* and hookworm infection when children of wastewater farmers and regular farmers were compared (Feenstra et al, 2000).

A study conducted in the city of Faisalabad in Pakistan where farmers were using untreated wastewater with a mean *Ascaris* concentration of 142 eggs/litre and a mean hookworm concentration of 558 eggs/litre (Ensink et al., 2004b) showed, as compared to the Haroonabad study, a much lower prevalence of hookworm infection of less than 15% among wastewater users (Ensink et al., 2004a). The studies in Faisalabad and Haroonabad showed similar outcomes, with an almost six-fold increased risk of hookworm infection in adult farmers, but no additional risk in children. *Ascaris* infections in both adults and children were low and showed no significant additional risk when compared to regular farmers (Ensink et al., draft).

The findings in Pakistan are in contrast to outcomes of research undertaken in Mexico, where children of wastewater farmers had an over five and twelve fold increased risk of *Ascaris* infection (Cifuentes, 1998; Blumenthal et al., 2001). The same studies in Mexico compared *Ascaris* prevalence in wastewater farmers using untreated (125 nematode eggs/litre) wastewater and wastewater conform (< 1 nematode egg per litre) the 1989 WHO water quality guidelines and showed clearly that with deteriorating water quality the risk of *Ascaris* infections increased (Cifuentes, 1998; Peasey, 2000; Blumenthal et al., 2001).

In Mexico wastewater retained in a reservoir before it was used in irrigation resulted in an average nematode concentration of less than 1 nematode egg/litre and was thus of WHO standards. Farmers using this water had similar (low) levels of *Ascaris* infections, compared with farmers using rain water, though an increased risk of *Ascaris* infection was found in children of wastewater farmers (Peasey, 2000 and Blumenthal et al., 2001).

## 2.7 HEALTH RISKS

A third source of potential contamination is found in the manure used by farmers in the management of soil fertility. According to Sonou *et al*, (2001), vegetables cultivated with manure are highly infected by bacteria, indicating contamination from a faecal source. Poultry manure, which represents 75% of the organic fertilizer used, generally contains Faecal Coliforms (1.30.106/g) and Faecal Streptococci (3.4.106/g) (Westcott, 1997). Fresh poultry manure sometimes contains disease causing pathogens that can contaminate produce. Manure from swine and carnivores can contain helminths as well as other bacteria. Studies have shown that about 60% of farmers apply fresh poultry manure directly without composting while 40% heap it for some few weeks or more before use (Drechsel and Kunze, 2001)

The cost of pipe-borne water makes it unaffordable to farmers. The use of untreated wastewater for irrigation has therefore become a widespread practice, with its attendant health hazards .When wastewater is used for irrigation without any treatment the pathogens present are applied to the agricultural land. This poses a potential health risk to people exposed to it, such as field workers and their families, consumers and handlers of wastewater-irrigated crops and people living in the neighborhood, passing the fields frequently. The WHO in 1989 documented that the health hazards associated with direct and indirect wastewater use are of two kinds:

- The rural health and safety problem for those working on the land or living on or near the land where the water is being used, and
- The risk that contaminated products from the wastewater use area may subsequently infect humans or animals through consumption or handling of the foodstuff or through secondary human contamination by consuming foodstuffs from animals that used the area.

It has been recorded by the High Council of Water and Climate that wastewater reuse may lead to public health risks of transmission of enteric diseases following consumption of raw vegetables. It may also result in the transmission of endemic parasitic diseases that mostly strike children.

Studies have showed that the nutrients and microbiological contaminants in irrigation water sources, in most cases exceed the WHO guidelines significantly (Keraita *et al.,* 2003b). However, according to the World Health Organization, (WHO, 1989) the actual health risks (which is the risk of people falling ill) is lower than the potential health risk. The potential health risk is based on the number of pathogens in the wastewater, while the actual health risk depends on three more factors:

- The time pathogens survive in water or soil
- The dose in which pathogens are infective to a human host
- Host immunity for pathogens circulating in the environment.

The actual risks to public health that may occur through wastewater use can be divided into three broad categories, namely:-

- Those affecting consumers of the crops grown with wastewater (consumer risk),
- Those affecting the agricultural and pond workers who are exposed to the wastewater (worker risk) and
- Those affecting populations living near the wastewater use scheme (nearby population risk) (Strauss et al, 1990)

Farmers rarely wear protective clothing or take any protective measures when applying water, or pesticides for that matter. Some are aware of such measures but cannot afford them or give them little priority. No extension services are offered to farmers on irrigation practices, related protection, etc.

**Table 2.1 Health risk from use of wastewater in agriculture**

| Type of pathogen/infection | Relative excess of frequency of infection or disease |
|---|---|
| Intestinal nematode infections (*Ascaris lumbricoides, Trichuris Trichura,* hookworm) | High |
| *Bacterial infections* | |
| Bacterial diarrheas (E.g. cholera, typhoid) | Lower |
| *Viruses* | |
| Viral diarrheas | |
| Hepatitis A | Lowest |

*Source: World Health Organization 1989*

The highest health risk is theoretically for helminth infections. Compared with other pathogens, helminths persist for long periods in the environment from a few months up to 30 years. Host immunity ranges from low to non-existent and the infective dose is small. The typical pattern of infection is one of the chronic rather than transient illnesses, with gradual increase in "worm load" (Strauss *et al*, 1990)

In Eritrea, the assessment of the health impacts of raw domestic sewage for vegetable cultivation showed heavy contamination of vegetables by fecal coliforms and *Giardia* cysts as well as other pathogenic bacteria such as *Shigella* and *Salmonella.* (Srikanth *et al*, 2004). It is known that dietary intake of raw greens (lettuce, cabbage) grown on the raw sewage appears to cause giardiasis, amebiasis, and diarrhoea in the farming community as well as in the surrounding area. Studies of wastewater usage in different parts of the world reveal various gastrointestinal problems in farming communities who are involved in this practice (Cifuentes *et al*, 1993). In addition to the risk of contamination by direct exposure, consumption of undercooked/ raw vegetables also poses a risk to health (Kowal *et al*, 1985).

The negative effects of this practice may include microbial contamination of the produce, health hazards to community residents consuming vegetables and raw salad greens, and occupational hazards to farm workers and consumers.

## 2.8 WASTEWATER USE GUIDELINES

The public health concerns related to wastewater use in agriculture have led to the development of sanitary guidelines that put restrictions on wastewater use according to its pathogen load. Two main "guideline" schools of thought can currently be distinguished. The American guidelines are based on the principle that there should be no risk as a result of wastewater use; therefore they promote the removal of all pathogenic organisms before wastewater can be used (USEPA, 1992). The World Health Organisation guidelines are based on the principle of no excess disease in the population using wastewater or consuming produce grown on wastewater (WHO, 1989), and its guidelines are therefore more lenient.

Both approaches have their critics, with the WHO being accused of basing its guidelines on partial studies, ignoring acquired immunity, and measuring with double standards (Shelef, 1991). The American guidelines though have been accused of being too strict and the wastewater treatment technology to guarantee these standards therefore too expensive. Shuval et al. (1997) estimated that bringing wastewater from WHO to USEPA guidelines would come at the cost of approximately US$ 3,500,000 – US$ 35,000,000 per prevented case of enteric disease in the exposed community. The less strict guidelines and the wider range of risk reduction, other than wastewater treatment, make the WHO guidelines more suitable for adoption in developing countries.

A first meeting of experts on the use of wastewater in aquaculture and agriculture was held by the WHO in 1971, which led to the development of the first guidelines (WHO, 1973) and an initial water quality standard of 100 faecal coliforms per 100 ml for crops consumed uncooked. A second meeting in 1987 lead to the inclusion of an intestinal nematode water quality standard (≤ 1 egg/litre) and a revision of the faecal coliform standard for crops eaten uncooked (≤ 1000/100 ml) (WHO, 1989). A review by a group of researchers in 2000, based on existing epidemiological evidence, resulted in a proposal for revision of the 1989 guidelines (Blumenthal et al, 2000) and the need to adopt a stricter nematode guideline value of less then 0.1 eggs/litre (annex 1). However others have suggested the guideline value could be increased to 10 eggs per litre (Ayres et al., 1992). The WHO wastewater irrigation guidelines are currently under review with new guidelines expected in 2004.

The four main measures put forward by the World Health Organization to protect health when wastewater is used are: wastewater treatment, crop restriction, control of wastewater application and human exposure, control and promotion of hygiene (WHO, 1989). Depending on the local sociocultural, institutional and economic conditions, a combination of measures could be selected

**Table 2.2 Recommended microbiological quality guidelines for treated wastewater used for crop irrigation[a]**

| Category | Reuse conditions | Exposed group | Intestinal nematodes[b] (arithmetic mean no. of eggs per litre[c]) | Faecal conforms (geometric mean no. per 100 ml[c]) | Wastewater treatment expected to achieve the required microbiological quality |
|---|---|---|---|---|---|
| A | Irrigation of crops likely to be eaten uncooked, sports fields, public parks | Workers, consumers, public | <1 | <1000[d] | A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment |
| B | Irrigation of cereal crops, industrial crops, fodder crops, | Workers | <1 | No standard recommended | Retention in stabilization ponds for 8-10 days or equivalent helminth and faecal |

| | | | | | |
|---|---|---|---|---|---|
| | pasture and trees[e] | | | | coliform removal |
| C | Localized irrigation[f] of crops in category B if exposure of workers and the public does not occur | None | Not applicable | Not applicable | Pretreatment as required by the irrigation technology, but not less than primary sedimentation |

*Source:* World Health Organization (1989).

[a] In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account, and the guidelines modified accordingly.
[b] *Ascaris* and *Trichuris* species and hookworms.
[c] During the irrigation period.
[d] A more stringent guideline (<200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.
[e] In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.
[f] Also called drip or trickle irrigation.

## 2.9 PATHOGENS AND PARASITES IN WASTEWATER

Water plays a major role in mobilizing and transporting microorganisms. Rainfall washes organisms from faeces or vegetation surfaces and directs them into soils or along the land surface into surface water. Several pathogenic microbes and parasites are commonly found in wastewater, these pathogens could be bacterial, opportunistic bacterial pathogens, antibiotic producing bacteria, viral pathogens as well as protozoan parasites and helminthes.

### 2.9.1 Bacterial Pathogens

Faecal matter contains up to $10^{12}$ bacteria per gram which constitutes approximately 9% by wet weight of the faeces (Dean and Lund, 1981). Coliform contamination levels of vegetables are often almost the equivalent of a similar amount of fresh faeces (Keraita *et al.,* 2003b). Wastewater bacteria have been characterized and belong to the following groups (Dott and Kamper, 1988):

- Gram-negative facultative anaerobic bacteria(e.g., *Aeromonas*, *Klebsiella*, *Esherichia,Enterobacter*, And *Shigella*)
- Gram-negative aerobic bacteria (e.g., *Pseudomonas*, *Flavobacterium* And *Acinetobacter*)
- Gram-positive spore-forming bacteria(e.g., *Bacillus* spp)

- Non-spore-forming gram-positive bacteria (e.g.,*Corynebacterium*, *Arthrobacter*)

These pathogens cause enteric infections such as typhoid fever, cholera, and shigellosis. *Salmonella* are the most predominant pathogenic bacteria in wastewater and they cause typhoid and paratyphoid fever and gastroenteritis. Their numbers in wastewater range from few to 8,000 organisms per 100ml (Feachem *et al*, 1983).

### 2.9.2 Protozoan Parasites

The major waterborne pathogenic protozoa affecting humans are *Giardia lamblia*, and *Cryptosporidium.* According to Dawson (2005) protozoan parasites enter the food production process via three main routes:

- Through contamination of food ingredients or raw materials on the farm;
- Through contaminated water included in the final product processing or washing, or used for cleaning processing equipment;
- Through transfer or spread via infected food handlers or food preparers in production, food service or domestic settings.

### 2.9.3 Helminth Parasites

Although helminths are not generally studied by microbiologists, their presence in wastewater, along with bacterial and viral pathogens and protozoan parasites, is of great concern in regards to human health.

The term "helminth" refers to a variety of worms that live as parasites in many species of animals and the human body. They belong to three biological categories: - trematodes (flukes), nematodes (roundworms) and cestodes (tapeworms). *Ascaris lumbricoides, Necata americanus* and *Ancylostoma dueodenale and Trichuris trichura* are all helminths belonging to the phylum nematode (Bogtish, 1998). Their ova are excreted in faeces and spread by wastewater, soil, or food.Helminthes infection occurs when worms (or eggs) enter, mature, lay eggs and feed off a person. Helminthes infections include soil borne intestinal nematodes such as roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichura*) and hookworm, and water borne species such as *Shistosoma haematobium* and *S. mansonii.*

Helminthes infections affect over a quarter of the world's population, especially in the developing countries. These long-lasting parasitic infections cause widespread immune activation and dysregulation, a dominant $Th_2$ cytokine immune profile and an immune hyporesponsiveness state (Barkow *et al*., 2001). The names of the important helminthes, where they live in the human body and their geographical distribution are presented in ‘Important Helminthes in School Children’ (UNESCO media services, 2002).

They affect the health and well-being of millions of people, especially young people. About 400 million school-age children are infected by roundworm, whipworm, hookworm, shistosomiasis and other flukes and/or guinea worm. These parasites consume nutrients from the children they infect. In doing so they bring about or aggravate malnutrition, weaken the immune system and retard children's physical and mental development.

Whipworm infections are associated with a high incidence of dysentery, chronic colitis, anaemia and growth retardation (UNESCO media services, 2001). Hookworm infections cause iron deficiency anaemia. Even minor hookworm infections can result in severe anaemia in children and in adolescent girls. Iron deficiency anaemia has been associated not only with decreased physical development, but also with decreased mental development. Helminthes infections are a leading cause of diseases among young people and adults in the world today. Helminthes infections can have a significant negative impact on school enrolment, attendance, and even the ability to learn.

The parasites that cause helminthes infections are naturally present in the environment. However the high prevalence of intestinal parasite infections in certain parts of the world is closely correlated with poverty and poor environmental hygiene. Most commonly, people become infected with helminthes by coming into contact with soil, water or food that contains the eggs or young worms of these parasites.

It has been successfully proven that it takes only a single helminthes egg to cause an infection in an individual (Schwartzbrod, 1998). Helminthes destroy the tissues and organs in which they live; causing abdominal pain, diarrhoea, intestinal obstruction, anaemia, ulcers, and various other health problems. If treatment is not given in time, heavy or long-term infection with various helminthes can result in death (UNESCO Media services, 2002).

Soil-transmitted helminthes (STH) infections are endemic in communities where poor environmental sanitation and poor personal hygiene are prevalent, as occurs in the majority of developing countries (Yodmani *et al.,* 1982). Yu *et al*. (1993) showed that environmental pollution, sanitary condition and human behaviour play an important role in the transmission of STH infection. Yodmani *et al*. (1982) indicated that many sources of ascariasis from the host and in the environment such as soil in the shantytowns and vegetables sold in the market resulted in continuous active transmission of ascariasis in the area.

Another study was carried out on sewage farms, streams and vegetables to determine the sources and routes of STH infection in Sanliurfa, Turkey. Stool samples from farmhouse inhabitants as well as soil and vegetable samples from the gardens were collected and examined. In addition, water samples from streams and vegetable samples from the city market were collected and examined. One hundred and eighty-seven (59.5%) of a total of 314 samples, including 88.4% of the stool samples, 60.8% of the water samples, 84.4% of the soil samples and 14% of the vegetable samples, were found to be positive for STH eggs. These results indicate that the water, soil and vegetables are heavily contaminated, and suggest a vicious circle between humans and the environment. Improving environmental sanitation is imperative for the control of soil-transmitted helminthiasis.

Helminthes parasites pass out of infected individuals in faeces and urine, so their numbers explode in areas where human waste is not properly managed. Fertilized eggs deposited in the soil develop rapidly and, depending on environmental conditions, may reach the infective stage within a matter of weeks (Klaas, 1987). Thereafter, eggs are transferred from soil to the vegetables then onto to hands and finally to the mouth (Kobayashi, 1999).

At a minimum, sanitary improvements required to reduce helminthes infections include: safe, efficient and hygienic management of water, safe, efficient and hygienic disposal of faeces, regular and effective use of water (with a scouring agent like soap) for hand washing after contact with stools; and hygienic food preparation methods (UNESCO media services, 2001).

All the above infections and resulting health problems can be prevented or greatly reduced through cost-effective interventions. Infections of the major intestinal helminthes can be prevented by avoiding ingestion of, or contact with, contaminated soil. Avoiding infested water greatly reduces shistosomiasis. Food safety measures help prevent fluke infections which are transmitted by meat or vegetables. Provision of safe water sources has been shown to be extremely effective in preventing guinea worm infection (UNESCO media services, 2001)

The soil-transmitted helminths are the most common and the most critical to human health. The ova are very resistant to environmental stress and to chlorination even in wastewater treatment plants (Little, 1986). The severity of helminth infections depends on the number of worms which invaded the body from outside. This worm load also determines the rate at which infection is propagated by transmission of eggs in the faeces, urine or sputum of the human host. Helminths eggs are also commonly found on such vegetables irrigated with wastewater or get transferred from the soil when it rains or during irrigation. When these contaminated vegetables are eaten raw or with inadequate cooking, the eggs burrow into the intestines and move to other sites and cause diseases. However, the survival of these organisms varies with different environmental factors e.g. low temperature which enhances the longer persistence of the pathogen on the crops.

**Table 2.3 Survival times of selected excreted pathogens (helminthes) on crops**

| Helminths | Survival time |
|---|---|
| *Ascaris lumbricoides* | 30 days |
| Hookworm | 60 but usually 30days |
| *Taenia* spp/*Trichuris* | 60 but usually less |

Source: (WHO, 1989; summerised by Feachem et al., 1983)

#### 2.9.3.1 Hookworm

The hookworm is a nematode parasite that lives in the small intestine of its host, which may be a mammal such as a dog, cat, or human. Two species of hookworms commonly infect humans, *Ancylostoma duodenale* and *Necator americanus*. The geographical distribution of these two species significantly overlaps. *Necator americanus* predominates in the Americas, Sub-Saharan Africa, Southeast Asia, China and Indonesia, while *A. duodenale* predominates in the Middle East, North Africa, India and (formerly) in southern Europe. Hookworms are thought to infect 800 million people worldwide.

Hookworms are much smaller than the large roundworm, *Ascaris lumbricoides*. The most significant risk of hookworm infection is anemia, secondary to loss of iron (and protein) in the gut. The worms suck blood and damage the mucosa. However, the blood loss in the stools is occult blood loss (not visibly apparent).

They are the leading cause of maternal and child morbidity in the developing countries of the tropics and subtropics. In susceptible children hookworms cause intellectual, cognitive and growth retardation, intrauterine growth retardation, prematurity and low birth weight among newborns born to infected mothers. Hookworm infection is rarely fatal, but anemia can be significant in the heavily infected individual.

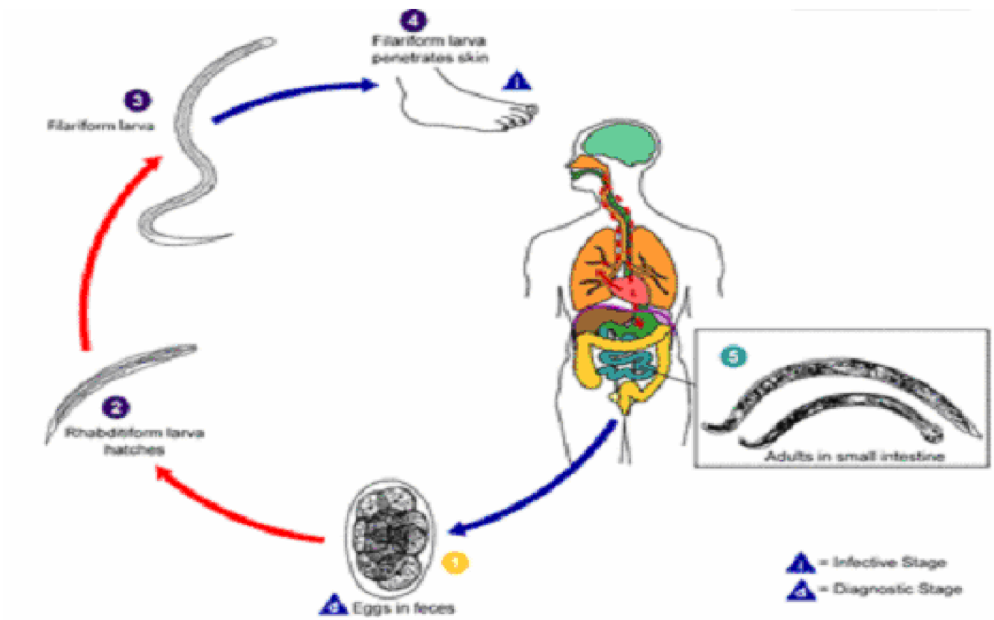



**Fig. 2.1 Life Cycle and Transmission of Hookworm**

source:http://www.dpd.cdc.gov/dpdx

### 2.9.3.2 ***Ascaris lumbricoides***

*Ascaris lumbricoides*, a parasitic round worm causes Ascariasis a debilitating human disease. Perhaps as many as one quarter of the world's people is infected, and ascariasis is particularly prevalent in tropical regions and in areas of poor hygiene. Other species of the genus *Ascaris* are parasitic and can cause disease in domestic animals.

Infection occurs through ingestion of food contaminated with fecal matter containing Ascaris eggs. The larvae hatch, burrow through the intestine, reach the lungs, and finally migrate up the respiratory tract. From there they are then reswallowed and mature in the intestine, growing up to

30 cm (12 in.) in length and anchoring themselves to the intestinal wall. Infections are usually accompanied by inflammation, fever, and diarrhea, and serious problems may develop if the worms migrate to other parts of the body.

Roughly 1.5 billion individuals are infected with this worm. Ascariasis is endemic in the United States, China, Ozark Mountains; Southeast Asia, central Africa and the coastal regions of the West Africa.

Ascariasis sources can often be measured by examining food for ova. In one field study in Marrakech, Morocco, where raw sewage is used to fertilize crop fields, Ascaris eggs were detected at the rate of 0.18 eggs/kg in potatoes, 0.27 eggs/kg in turnip, 4.63 eggs/kg in mint, 0.7 eggs/kg in carrots, and 1.64 eggs/kg in radish (Habbari *et al*, 1999). A similar study in the same area showed that 73% of children working on these farms were infected with helminths, particularly Ascaris, probably as a result of exposure to the raw sewage.

Roundworm infections can retard growth. They decrease the absorption of nutrients that the body needs to grow. They cause structural problems in the small intestine in children and are thought to be a cause of frequent or serious pulmonary disease among children. Intestinal obstructions frequently result in the hospitalization of children. Death is common in children when worms move to organs outside of the intestines such as the trachea, liver, and heart, or when complications occur.

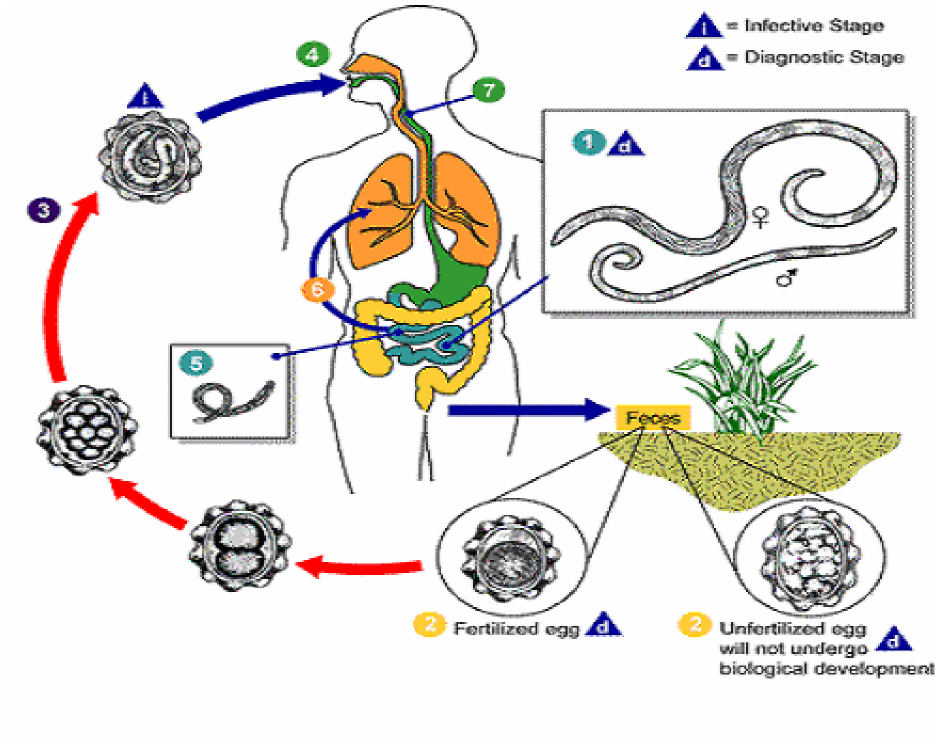



**Fig. 2.2 Life Cycle and Transmission of *Ascaris lumbricoides***

source:http://www.dpd.cdc.gov/dpdx

Adult worms (1) live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the feces (2). Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks (3), depending on the environmental conditions (optimum: moist, warm, shaded soil). After infective eggs are swallowed (4), the larvae hatch, (5) invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs. The larvae mature further in the lungs (6) (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed (7). Upon reaching the small intestine, they develop into adult worms (8). Between 2 and 3 months are required from ingestion of the infective eggs to oviposition by the adult female. Adult worms can live 1 to 2 years.

#### 2.9.3.3 *Strongyloides stercoralis*

This 2 mm-long worm is found both free-living in soil, and as a parasite of humans, other primates, and dogs. The free-living male and female worms are not infectious; however, their offspring all develop to infectious larvae. These infectious larvae penetrate the skin when there is contact with the soil. Some of them enter the superficial veins and ride the blood vessels to the lungs, where they enter the alveoli. They are then coughed up and swallowed into the gut, where they parasitise the intestinal mucosa (duodenum and jejunum).There, they reach sexual maturity and produce eggs (all infectious larvae are female and thus all parasitic adults are females). The eggs hatch in the intestine and young larvae are then excreted in the feces. It takes about two weeks to reach egg development from the initial skin penetration. By this process, S. stercoralis can cause both respiratory and gastrointestinal symptoms. Adult worms can live up to a year in dogs.

Autoinfection can also occur when the larvae which hatch from the eggs in the small intestine have time to mature to infectious larvae before being excreted. They penetrate the wall of the lower ileum or colon or the skin of the perianal region; enter the circulation again, up to the lungs, and back down to the small intestine thus repeating the cycle. Because of autoinfection, humans have been known to still be infected up to 50 years after they were first exposed to the parasite. Immunosuppressive drugs, such as those used for tissue transplantation, (especially corticosteroids) can increase the rate of autoinfection to the point where there is an overwhelming number of larvae migrating through the lungs, and in many cases this can prove fatal. While there are a number of drugs which will kill the adult worms, these drugs have little effect on the majority of these auto infective larvae during their migration through the body.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 STUDY AREA

Kumasi is the second largest and one of the fastest growing urban cities in Ghana with an estimated population of 1.2 million and an annual growth rate of 2.6% (Ghana Statistical Service, 2000). It lies between latitude 6° 42 North and longitude 1°35 West and an altitude of 287m. It covers a total area of 57sq.m and the topography of the region varies from gently undulating to distinctly hilly and mountainous (Taylor, 1982). The region has two major seasons, the rainy and dry seasons. The rainy season experiences major rains between March and July and minor rains between September and November with an annual rainfall of about 1300mm. The relative humidity ranges between 1270 to 1410 mm with average daily sunshine durations ranging between 2 to 7 hours and daily minimum and maximum temperatures of 21.20°C and 35.50°C, respectively (Meteorological Services Department, Kumasi Airport Weather Station, 2002)

Kumasi's unique position and availability of infrastructure coupled with the large markets have made it the hub of trade in the Ashanti region. The Kumasi Central Market, one of Africa's largest markets is in the region. Other markets including the Asafo market, the second largest in the region and the European market receives thousands of people daily.

The study was conducted on selected vegetable production (farms) sites, vegetable selling markets and street food vendor sites in and around Kumasi.

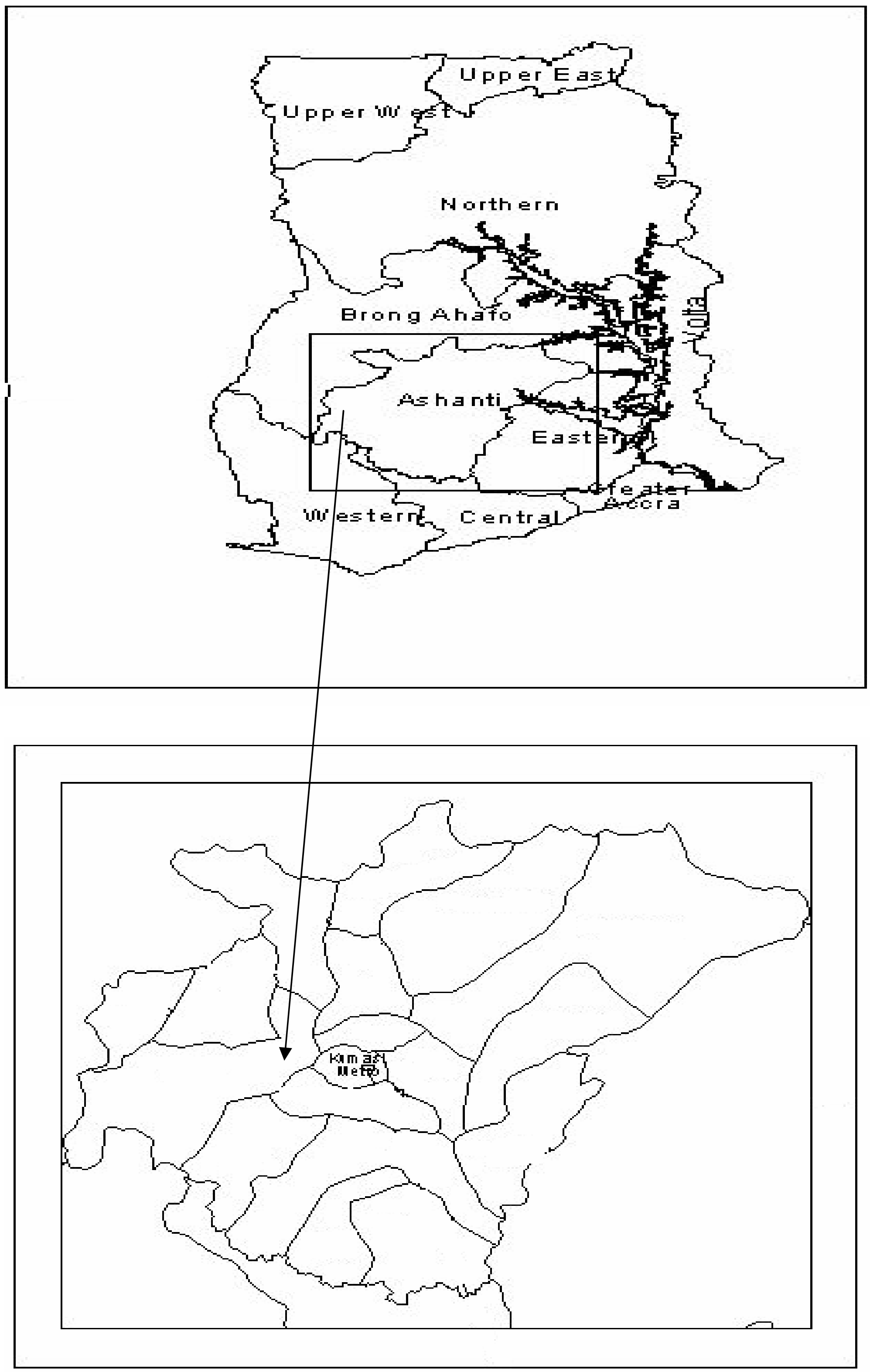



**Figure 3.1 Map showing Kumasi in relation to the rest of Ghana**

## 3.2 RESEARCH METHODS

### 3.2.1 Sampling sites

#### 3.2.1.1 Production (Farm) Sites:

Three production (farm) sites, two urban (Karikari and Badu farms) and one peri-urban farm at Deduako were selected for the study (Fig.3.2)

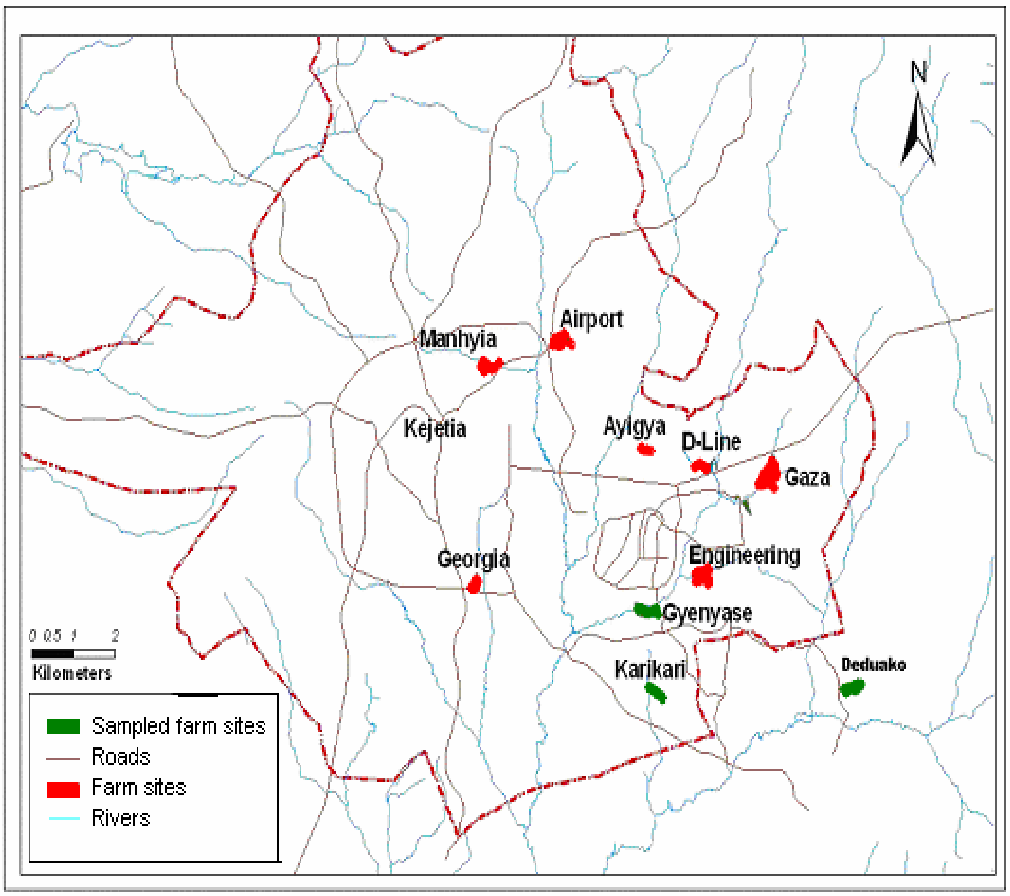



**Figure 3.2: Map of Urban/Peri Urban Vegetable Farm Sites in Kumasi and Selected Study Farms in Green Colour**

**SITE 1: KARIKARI FARM**

**Karikari Farm** is located close to Gyenyase, an urban suburb of Kumasi. It covers a total area of 3ha with 2.8ha being irrigated and an average bed size of 14sq.m. The land, which is in a low land area, belongs to the Chief of Gyenyasi. Although the farm is close to a stream, farmers do not use it for irrigation but rather obtain water from shallow wells dug at designated points on the farm and channeled along the ridges of the vegetable beds. Irrigation is done by means of watering cans.

Crops grown on this farm include cabbage, lettuce, green pepper, cassava and plantain.

**Plate 3.1: A section of Karikari Farm with some areas developed for residential use.**

**SITE 2: BADU'S FARM**

**Badu's Farm** is located at Gyenyase and is close to Kwame Nkrumah University of Science and Technology (KNUST) main sewerage treatment plant which is about 1km away from the university campus. The land which is owned by KNUST, covers a total area of 12.7ha with 10.3ha being irrigated and has an average bed size of 14sq.m. Farmers obtain irrigation water from shallow wells dug along the ridges of the beds and at designated points on the farm; irrigation is done by means of watering cans. Types of crops grown include lettuce, cabbage, spring onions, green pepper and carrots.

(a)

(b)

**Plate 3.2 (a&b) Sections of the Badu farm site**

**SITE 3: DEDUAKO**

The **peri-urban farm** at Deduako is located 10km from the centre of Kumasi. The farm lies in a low land area with water available all year round. It covers a total area of about 10ha with 9.3ha being irrigated. Crops grown include lettuce, cabbage, spring onions and carrots. Irrigation is mainly overhead with motorized sprinkler using water from an impounded stream running close to the farm.

**Plate 3.3: A section of the Deduako farm site.**

### 3.2.1.2 Market Sites

Three market sites where produce from the selected study farms are sold were identified as part of the study. Produce from Karikari's farm were traced to the Railway market, those from Badu's farm to the European market and French line and produce from Deduako were traced to the Asafo market. Three sellers were selected from each market site for the study.

#### 3.2.1.3 Street Food Vendor Sites

The Kumasi Metropolitan Authority is divided into four sub-metros for ease of administration. Based on these divisions, five areas (Stadium, Oforikrom, Dichemso, Krofrom and Tech-Junction) were randomly selected for the study. Fifteen street food vendor sites that purchase lettuce from the selected study market sites were selected from these areas and included in the study.

### 3.2.2 Sampling

Sampling was done between June and December, 2005 and between February and June, 2006 at all the selected farms, markets and street food vendor sites.

A total of 321 samples were analysed. Out of this number was 162 lettuce samples from all three production(farm) sites ( 54 lettuce heads per farm site), 90 lettuce heads from markets selling produce from the selected farm sites ( 27 from railways, 27 from Asafo and 36 from European and French line markets), 30 lettuce samples from selected street food vendors, 21 irrigation water samples from the selected farms (9 from Karikari, 9 from Badu and 3 from Deduako) and 18 refreshing water samples from Railways and European/French line markets. Sellers at Asafo market displayed lettuce without refreshing water.

#### 3.2.2.1 Lettuce Sampling from Production (Farm) site

On each sampling date, three lettuce heads per bed were randomly harvested from six beds under cultivation per farm site by means of a sketch map and placed separately in labeled sterile plastic bags.


**Plate 3.4: Sampling of lettuce from a bed at one of the selected farm sites**

#### 3.2.2.2 Market

From each market seller, three lettuce heads were collected around mid-day to ensure that the lettuce had experienced some splashing/refreshing with water. Each was placed separately in separate labeled plastic bags.

#### 3.2.2.3 Street Food Vendors

From each street food vendor site, about 120g of already prepared and sliced lettuce were collected and placed in labeled food bags.

#### 3.2.2.4 Irrigation/Refreshing Water Sampling

On each sampling date, irrigation water samples from either the hand dug shallow wells or streams on the farms were collected using one litre Duran Schott bottles. The bottles were dipped into the water without opening until it was about 30cm below the water surface. The bottle was then opened and filled; the cap was replaced under the water and tightened.

Samples of splashing/refreshing water used for the lettuce by the sellers in the market were also taken aseptically from the selling receptacles using sterile one liter Duran Schott bottles.

All samples (lettuce, irrigation and refreshing water) were transported to the laboratory in an ice chest containing ice packs and analysed for protozoan parasites and helminth eggs within 24hours.

### 3.2.3 Preparation of Samples and Quantification of Helminth Eggs

One hundred grams of lettuce was washed under running water into a bowl, and then transferred into a sterile container. The water was allowed to settle in a 2 litre container for 3hours or overnight. As much supernatant as possible was sucked up using a vacuum pump and the sediment placed into tubes and centrifuged for 3 min at 400g. The supernatant was poured off and the sediment resuspended with Zinc Sulphate of 1.3 densities and homogenized with a spatula. It was again centrifuged for 3 min at 400g. The Zinc Sulphate supernatant was poured into a fresh 2 litre bottle and diluted with 1 litre of water. The container was allowed to stand for 3 hours or overnight. As much supernatant was sucked up and the sediment resuspended by shaking and emptied into centrifuge tubes, the bottle was rinsed twice with deionized water and placed into the tubes with the sediment. The tubes were centrifuged for 3min at 480g. The sediments were regrouped into one tube and centrifuged again for 3min at 1600 rpm. The sediment was again resuspended in about 5ml acid/alcohol ($H_2SO_4$+$C_2H_5OH$) buffer solution and 2ml ethyl acetate solution. It was shaken and occasionally opened to let out gas. It was then centrifuged for 3min at 660g. Much of the supernatant was sucked up leaving less than 1ml of liquid. The deposits were read on a slide using a light microscope.

The number of eggs per liter was calculated from the equation:

$N = AX/PV$

Where N = Number of eggs per liter of 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)

### 3.2.4 Identification of Helminth Eggs

The helminth eggs were identified on the basis of their shape and size and compared with standard eggs on chart (WHO, 1996). The counting was done under a light microscope in both chambers of a haemocytometer.

### 3.2.5 Health Risk Assessment and Perception

Risk and perception were assessed using structured questionnaires, focus group discussions and an observation check lists at all stages of sampling (farm sites, markets and street vendor sites) to document prevailing hygienic practices. The specific health risks examined included irrigation practices, Personal Protective Equipment and processing procedures (handling, washing, serving, and preservation of leftovers).

## 3.3 STATISTICAL ANALYSIS

The statistical package for Social Scientists (SPSS) version 11.0 was used for testing the various statistical relationships between and within variables.

## CHAPTER FOUR

## 4.0 RESULTS

### 4.1 HELMINTH EGGS IN IRRIGATION WATER AND LETTUCE LEAVES AT PRODUCTION SITES

Morphological features of the different helminth eggs contained in the irrigation water samples from the different farm sites suggest that they are mostly, *Ascaris lumbricoides, Shistosoma, Hookworm, Trichuris trichura, Taenia, Clonorchis* and Strongyloides larvae (Table 4.1).

**Table 4.1 Description of helminth eggs/larvae associated with wastewater irrigated vegetables**

| WHO Description (Size, shape and colour) | Sample Description | Probable identification |
|---|---|---|
| The fertile eggs are oval to round, 55µm to 75µm 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, normal fertile eggs lack the mamillated layer and are referred to as "decorticated" eggs. The infertile eggs are elongated, much larger in size measuring 85-95 by 43 to 47µm wide. They have thin shells and grossly irregular mamillated layer, content of the egg is usually granular and lacks organization. | The eggs were brown in colour and oval/round in shape, measuring 50µm to 60µm in length and 35µm to 45 µm in width and having a thick lumpy outer shell. There was one cell inside the egg which is separated at both ends. Most of the eggs had conspicuous mamillations on their outer surface but few lacked the mamillated layer. (Plate 4.3) | *Ascaris lumbricoides* |
| These measure 50 to 55 by 22 to 24µm. They are elongated and lemon shaped | These eggs were elongated and lemon shaped with | *Trichuris trichura* |

| | | |
|---|---|---|
| with a brown, smooth shell, bipolar prominences (plugs) at each end. At the time the egg is laid, it contains a single-cell ovum | smooth outer shell and bipolar plugs at each end. They contained single-cell ovum | |
| These eggs are characteristically barrel-shaped with a thin, hyaline shell; they measure 65 to 75by 36 to 40µm. The egg is colourless with greyish 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 | The eggs were colourless barrel-shaped with thin, hyaline shell containing greyish cells in the 8 cell stage. | Hookworm |
| 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 | These measured from 115 to 130µm in length and 55 to 60µm in width. the eggs were elongated and rounded at the anterior end with a terminal spine at the posterior end. they contained miracidia (Plate 4.1) | *Shistosoma haematobium* |
| 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 | The larvae measured 150 to 250µm in length and 14 to 18µm in width. It had a conspicuous buccal capsule, an attenuated tail and a prominent genital primordium.(Plate 4.2) | *Strongyloides stecoralis* |
| 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 | The eggs were all identical, with thick walls, containing hooked embryo with a hyaline membrane around the eggs. | *Taenia* spp |

| | | |
|---|---|---|
| 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 | The eggs were smaller compared to the others. They were cup-shaped with operculum and small protuberance at the opposite end. They usually had adherent debris. | *Clonorchis* |

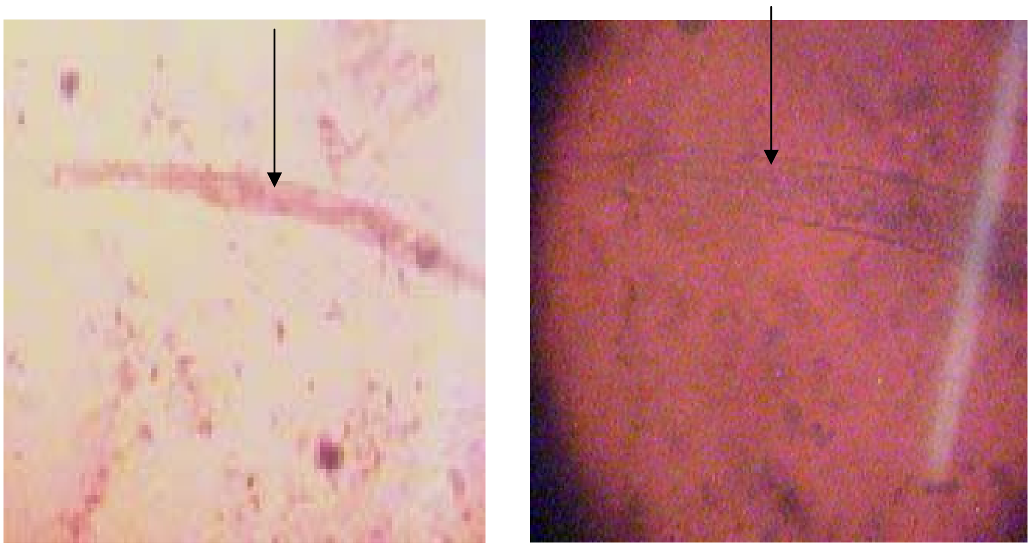


**Plate 4.1 Larvae of *Strongyloides stecoralis* (low and high magnifications)**

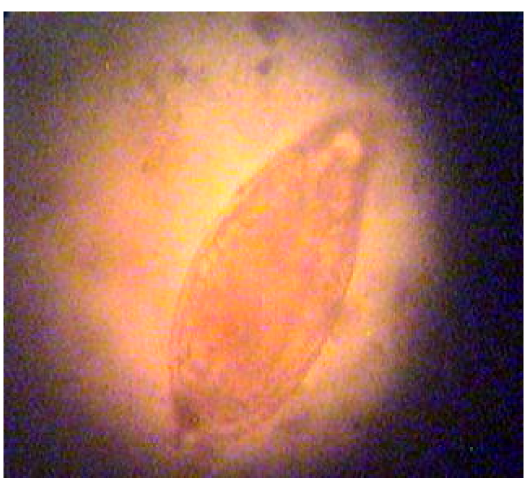


**Plate 4.2 *Shistosoma haematobium***

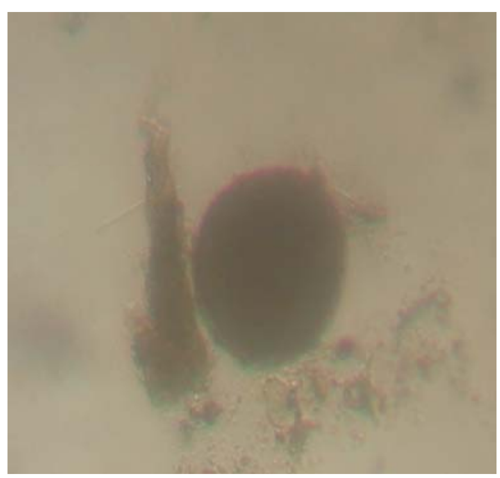


**Plate 4.3** *Ascaris lumbricoides*

Irrigation water sampled from Karikari, Badu and Deduako farms contained Helminths with mean total egg/larvae counts of 3, 25 and 3 eggs $l^{-1}$, respectively (Table 4.2). There were no statistically significant differences between Karikari and Deduako farms (P =0.215) but there were significant differences between Karikari and Badu ($P \leq 0.001$) and between Badu and Deduako ($P \leq 0.001$).

*Ascaris lumbricoides* eggs were contained in all the irrigation water samples examined and had the highest numbers ranging between mean counts of 2-17 eggs $l^{-1}$ compared to 0-4 larvae $l^{-1}$ for *Strongyloides*, 0-1 eggs $l^{-1}$ for *Shistosoma* and 0-1 eggs $l^{-1}$ for *Trichuris trichura*. However, Taenia, Hookworm and *Clonorchis* ranged between 0-1 eggs $l^{-1}$ for water samples (Table 4.2) (Figure 4.1).

**Table 4.2 Types and population densities (per litre) of helminth eggs in Irrigation water for lettuce production and on lettuce leaves (per 100 g) in three sites in Urban Kumasi**

| Farm sampling site | *Strongyloides*[1] | *Ascaris* | *Shistosoma* | *Hookworm* | *Trichuris* | *Taenia* | *Clonorchis* | Mean total egg count |
|---|---|---|---|---|---|---|---|---|
| Karikari farm | 1(1) | 2(4) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 3(5) |
| Badu's farm | 4(1) | 17(12) | 1(0) | 1(0) | 1(0) | 1(1) | 0(0) | 25(14) |
| Deduako farm | 0(1) | 3(3) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 3(4) |

Figures in parenthesis represent mean numbers of eggs/larvae on lettuce leaves

[1] These were larvae and not eggs

N for Lettuce =54 for all farm sites; n for Irrigation Water samples = 9 for Karikari and Badu farms and 3 for Deduako farm

Similarly, mean counts of helminth eggs on lettuce samples from the selected farm sites were high recording 5, 14 and 4 eggs $100g^{-1}$ wet weight for Karikari, Badu and Deduako farms, respectively (Table 4.2). These differences between the Badu and Karikari farms and between Badu and Deduako were statistically significant ($P \leq 0.001$) but differences between Deduako and Karikari, were not statistically significant ($P = 0.444$). *Ascaris lumbricoides* eggs on lettuce leaves were also high in all the three farm sites studied with a mean range of 3-12 eggs $100g^{-1}$. However, *Strongyloides* varied from 0-1 larvae $100g^{-1}$ and *Taenia* between 0-1eggs $100g^{-1}$ wet weight (Table 4.2) (Fig. 4.2).

Considering helminth egg population in irrigation water samples from all the three farms, percentage numbers in Badu's farm were highest, 81.3% (25 eggs $l^{-1}$), followed by Deduako, 9.9% (3 eggs $l^{-1}$) and Karikari, 8.8% (3 eggs $l^{-1}$). In all the farms, *Ascaris lumbricoides* eggs dominated with population density of 75%, 69% and 89% and Strongyloides larvae were least prevalent with 25%, 19% and 11% of the total helminth egg numbers counted on Karikari, Badu and Deduako farms, respectively. Other helminthes eggs identified on samples from Badu's farm included *Shistosoma* (3.2%), Hookworm (2.9%), *Trichuris trichura* (2.7), *Taenia* spp (2.7%) and *Clonorchis* (0.5%).

The percentage of helminth eggs on lettuce leave samples were similarly high, 58% (14 eggs $100g^{-1}$) in Badu, 22% in Karikari (5 eggs $100g^{-1}$) and 20% in Deduako (4 eggs $100g^{-1}$). Numbers of *Ascaris lumbricoides* eggs were also high on the lettuce leaves from all the three farms recording 74% in Karikari, 82% in Badu and 60% for Deduako.

The variety of helminth eggs observed in irrigation water from Badu's farm was wider (*Ascaris lumbricoides, Strongyloides, Shistosoma, Hookworm, Trichuris trichura, and Taenia)* compared to (*Ascaris lumbricoides* and *Strongyloides*) in Karikari and Deduako (Figure 4.1). Similarly, helminth eggs on lettuce leaves also varied widely on Badu's farm (*Ascaris lumbricoides, Strongyloides, Shistosoma haematobium, Hookworm, Trichuris, Taenia*, *Clonorchis*) compared to Karikari (*Ascaris lumbricoides, Strongyloides, Shistosoma haematobium, Trichuris, Taenia*) and Deduako (*Ascaris lumbricoides, Strongyloides, Shistosoma haematobium, Hookworm, Trichuris*) (Fig 4.2)

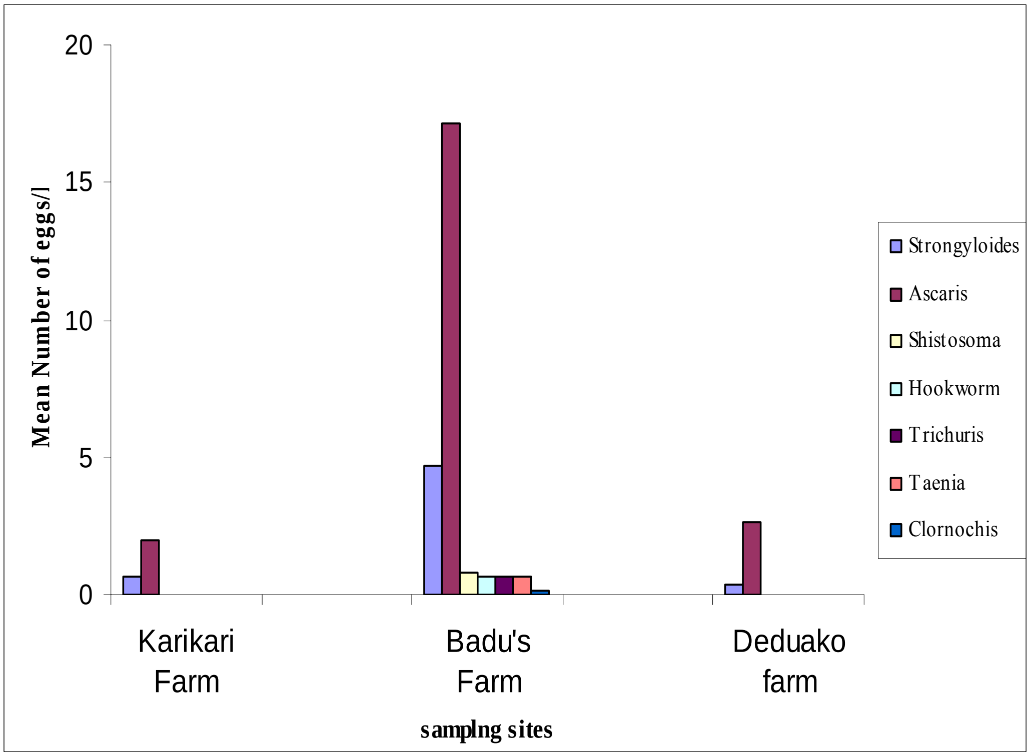



**Figure 4.1: Helminth eggs/larvae in irrigation water samples from the farm sites.**

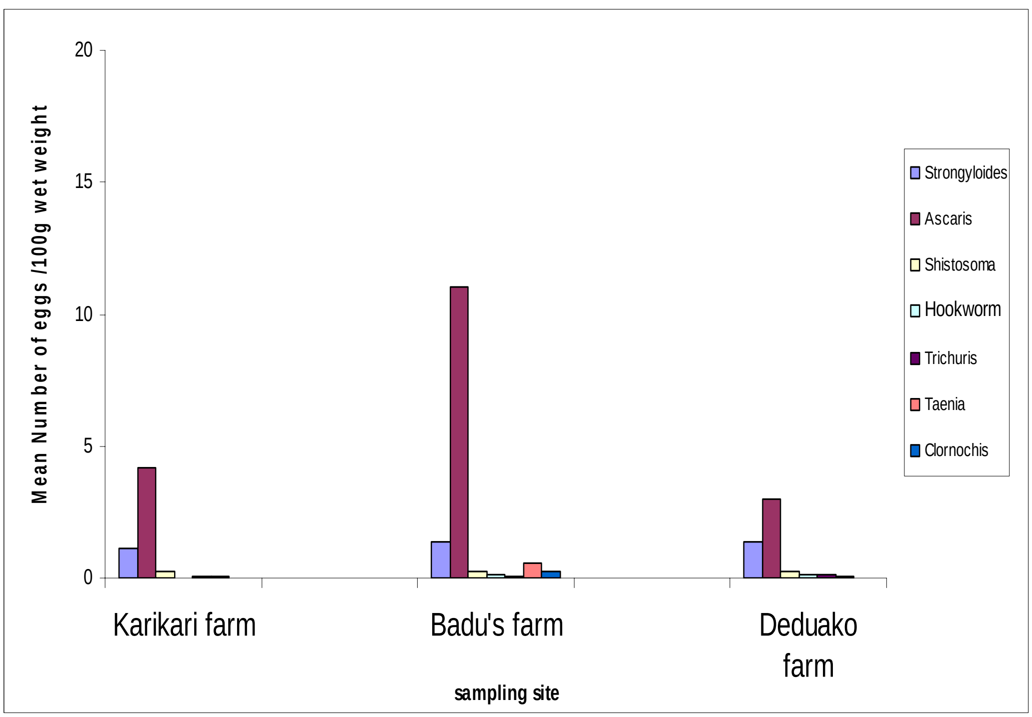



**Figure 4.2: Helminth eggs/larvae on lettuce samples from the farm sites.**

## 4.2 HELMINTH EGGS IN LETTUCE AND REFRESHING WATER FROM SELECTED MARKET SITES

Mean helminth eggs/larvae numbers on lettuce samples studied were 6, 7, and 2 eggs/100g wet weight from Railways, European/French Line and Asafo market sites, respectively (Table 4.3).There was no statistical difference (P =0.652) between the Railway market and the European/French Line market but the differences between the Railway and Asafo Market, and between Asafo market and European/French line markets, were statistically significant ($P \leq 0.001$).

Similarly, mean helminth eggs counts in refreshing water samples were 4 for Railways and 15 for European/French line markets (Table 4.3) and this difference was statistically significant ($P \leq 0.001$).

Compared to farm samples, most of the helminth eggs counted on lettuce leaves and in refreshing water in the markets were *Ascaris lumbricoides* with mean range of 1 to 6 eggs $100g^{-1}$ wet weight for lettuce leaves and 4 and 10 eggs $l^{-1}$ for refreshing water (Table 4.3).

**Table 4.3 Types and population densities (per litre) of helminth eggs in refreshing water for lettuce and on lettuce leaves (per 100 g) in three Market Sites in Urban Kumasi**

| Market sampling site | *Strongyloides*[1] | *Ascaris* | *Shistosoma* | *Hookworm* | *Trichuris* | *Taenia* | *Clonorchis* | Mean total egg count |
|---|---|---|---|---|---|---|---|---|
| Railways | 1(0) | 5(4) | 0 | 0 | 0 | 0 | 0 | 6(4) |
| European/ French Line | 1(4) | 6(10) | 0 | 0 | 0(1) | 0 | 0 | 7(15) |
| Asafo[2] | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |

Figures in parenthesis represent mean numbers of eggs/larvae in refreshing water [1] These are larvae and not eggs N for lettuce =27 for

Railways and Asafo Market and 36 for European/French Line market. N for refreshing water samples = 9 for Railways and 12 for European/French line [2]Asafo Market displays lettuce leaves without refreshing water.

Eggs of *Ascaris lumbricoides* were counted on almost all lettuce samples but represented 70% of all helminth eggs counted on lettuce from Railways (2-10 eggs $100g^{-1}$ wet weight), 78% from European/French Line samples (2-11 eggs $100g^{-1}$ wet weight) and 60% from the Asafo market (1-4 eggs $100g^{-1}$ wet weight) samples.

Similarly, helminth egg numbers in refreshing water were predominantly *Ascaris lumbricoides* and were 24% in the Railway samples ranging from 1 to 9 eggs $l^{-1}$, 76% in European/French Line samples ranging from 4 to 25 eggs $l^{-1}$.

There were no statistically significant differences in helminth egg numbers on lettuce between the Railway market and European/French line markets ($P = 0.652$) but in the refreshing water, these differences were statistically significant ($P \leq 0.001$).

The variety of helminth eggs observed in Refreshing water from the European/French line markets was wider (*Ascaris lumbricoides, Strongyloides, Shistosoma, Trichuris trichura, Clonorchis and Taenia)* compared to (*Ascaris lumbricoides, Shistosoma, Taenia* and *Strongyloides*) detected in samples from the railway market (Figure 4.3). Similarly, helminth eggs on lettuce leaves also varied widely on samples from the European/French line markets compared egg types detected on lettuce samples from the Railway and Asafo markets (Fig 4.4)

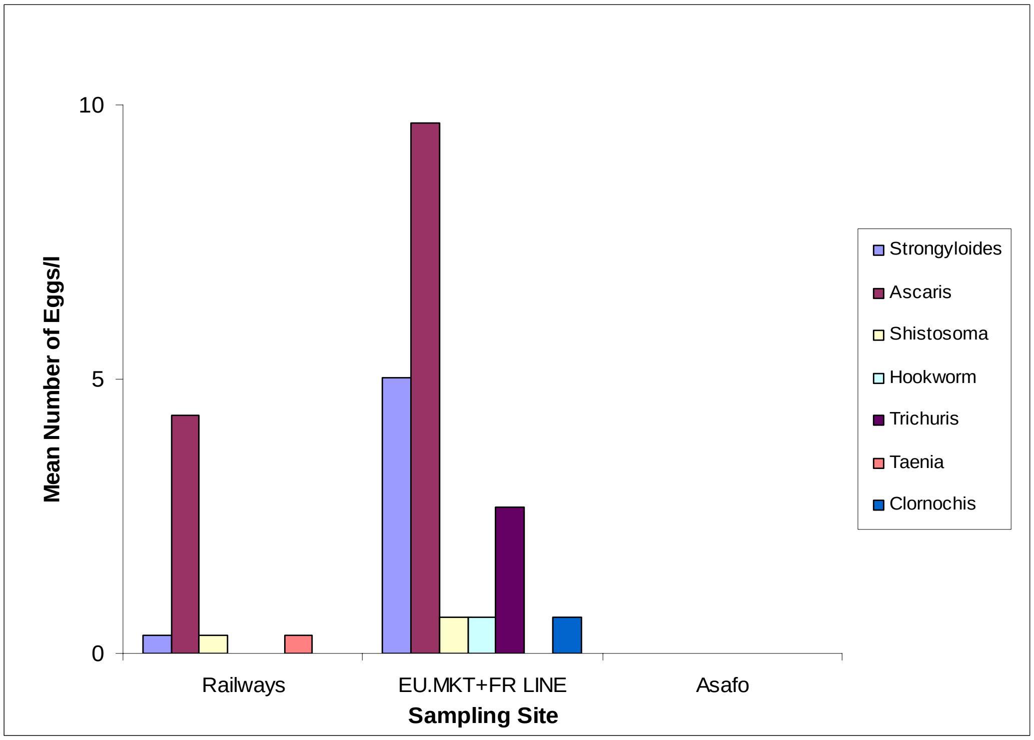



**Figure 4.3: Helminth eggs/larvae in refreshing water samples from the market sites.**

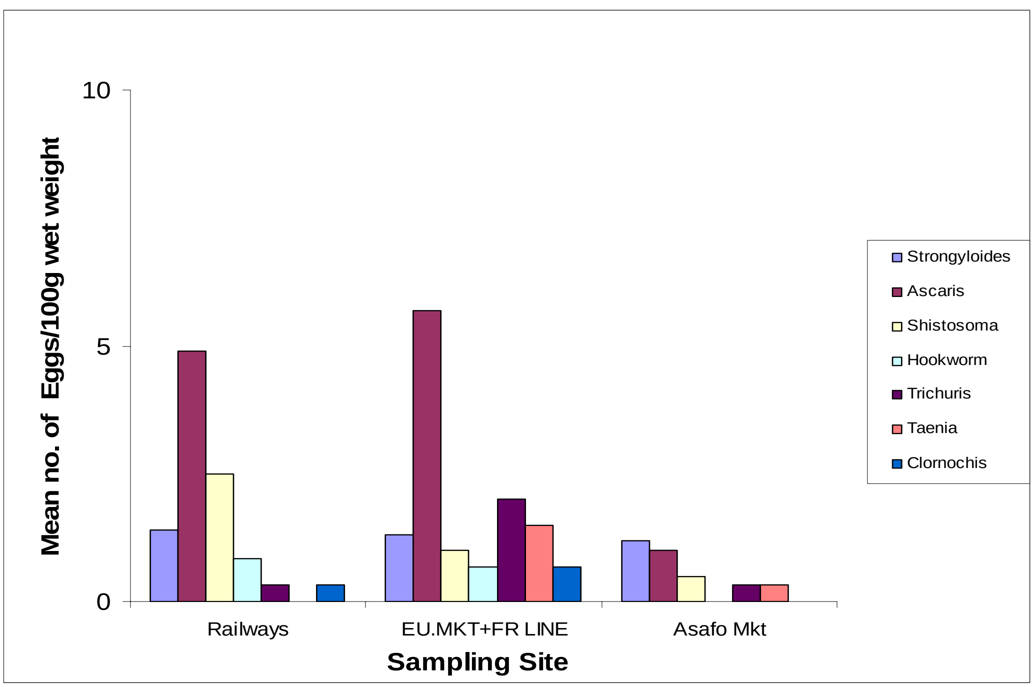



**Figure 4.4: Helminth eggs/larvae on lettuce samples from the market sites.**

## 4.3 RELATIONSHIPS BETWEEN HELMINTH EGGS/LARVAE COUNTS IN IRRIGATION WATER, FARM LETTUCE, REFRESHING WATER AND MARKET LETTUCE

Helminth egg counts on lettuce leaves from Karikari and Deduako farms was 25 to 36% more than counts from the irrigation water used. However, in Badu farm helminth egg numbers were 25% more in irrigation water compared to that on lettuce leaves (Fig.4.5). Comparing the numbers of helminth eggs on lettuce leaves and in irrigation water between the different farms, revealed weak positive correlations (r) of 0.437 for Karikari, 0.268 for Badu and 0.048 for Deduako.

In the same way, the population density of helminth eggs on lettuce leaves samples was 20% more on lettuce leaves on Railway market compared to the refreshing water used. However, the population counted from the European/French Line markets where 26% more in the refreshing water (Fig 4.6). A weak positive correlation exists between the lettuce leaves and refreshing water samples (r =0.285) for Railways and (r =0.211) for the European/French Line markets.

Along the production - market chain, helminth egg numbers either reduced or increased. From the Karikari farm to the Railway market, egg counts increased by 10.5% but from the Badu's farm to the European/French line markets they decreased by 33.3%. Egg numbers also decreased by 42.9% for produce from Deduako farm to the Asafo market (Fig 4.7).

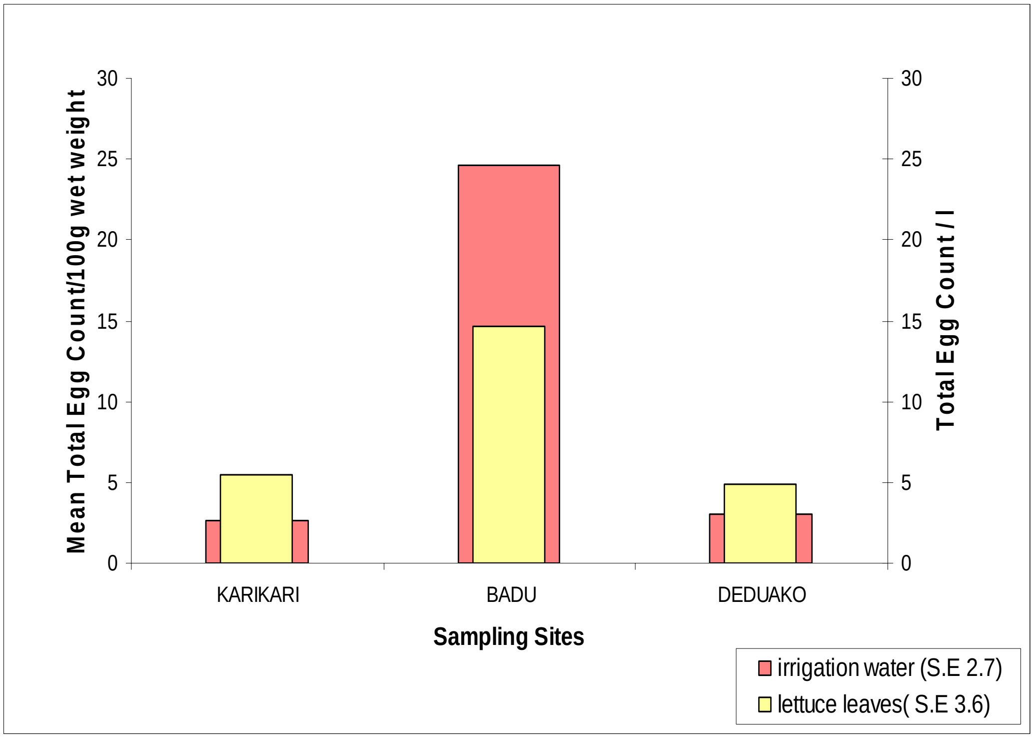



**Figure 4.5: Mean total count of helminth eggs on lettuce leaves and in irrigation water**

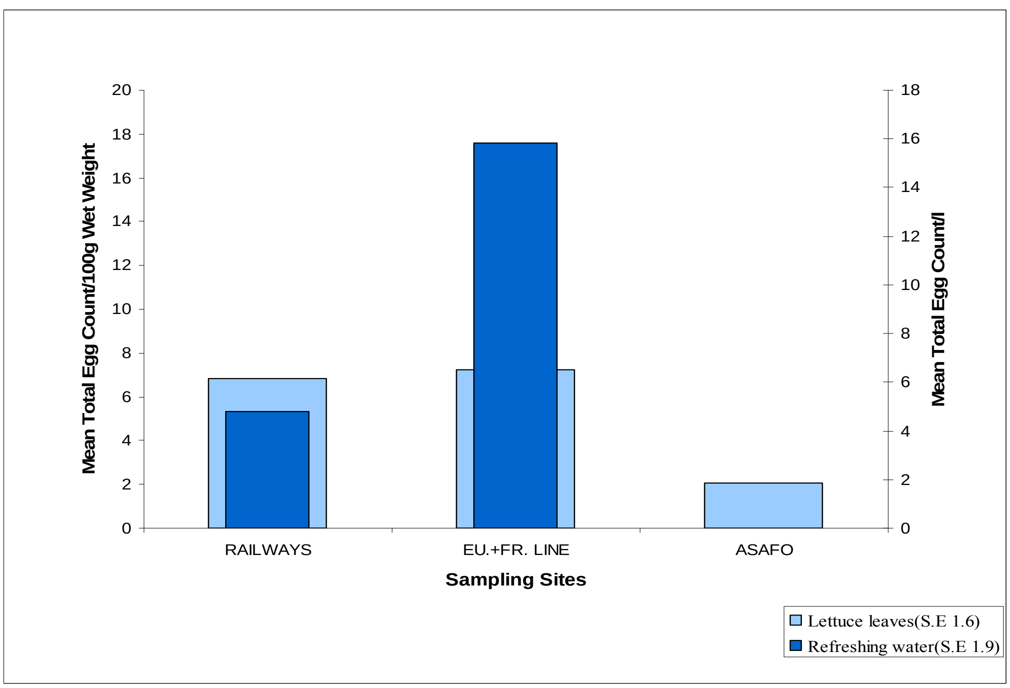
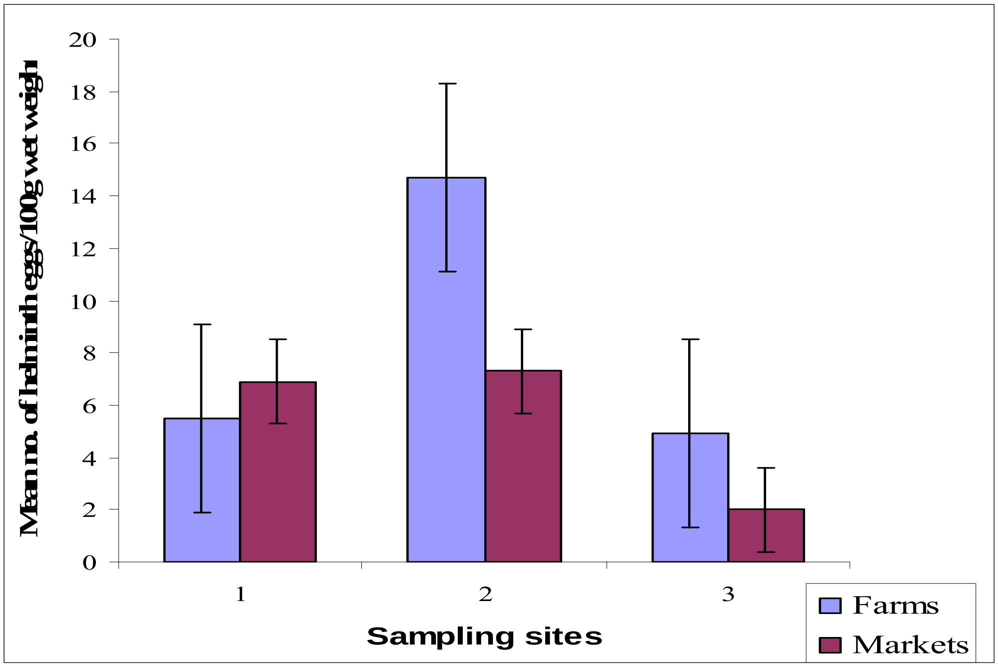



**Figure 4.6: Mean total count of helminth eggs on lettuce leaves and in refreshing water**




1: Karikari Farm –Railway Market, 2: Badu Farm – European/French line Market 3: Deduako Farm-Asafo Market

**Figure 4.7: Total helminth egg count on lettuce leaves on farm and their corresponding markets**

## 4.4 HELMINTH NUMBERS AT STREET FOOD VENDOR SITES

Sliced lettuce leaves sold as part of most ready to eat street foods were also found to carry helminth eggs. Mean counts were 2, 3, 1 and 2 eggs $100g^{-1}$ wet weight (Table 4.4) for Stadium, Krofrom, 'Tech Junction' and Dechiemso, respectively. *Ascaris lumbricoides* and *Shistosoma haematobium* were the two main microbial contaminants in all the samples analysed (Table 4.4) but the differences were not statistically significant (P =0.47). The total population density of helminth eggs on lettuce samples from Stadium ranged from 1 to 4 eggs $100g^{-1}$, *Ascaris lumbricoides* varied from 0 to 1 egg $100g^{-1}$ while *Shistosoma* ranged between 2 and 3 eggs $100g^{-1}$. Lettuce samples from Krofroum recorded the highest counts of helminth eggs with the total population density ranging from 1 to 7 eggs $100g^{-1}$ with both *Ascaris* and S*histosoma* recording a range of 1 to 4 eggs, respectively. The trend was no different from 'Tech Junction' and Dichemso where the population density range was from 1 to 3 eggs $100g^{-1}$wet weight, *Ascaris lumbricoides* and *Shistosoma* ranged from 0 to 1 and 1 to 2 and from 1to 2 and 0 to 1, respectively.

**Table 4.4 Mean number and types of helminth eggs on lettuce leaves (per 100g) from selected street food vendor sites.**

| Street food vendor site | *Ascaris lumbricoides* (range) | *Shistosoma haematobium* (range) | Mean total egg count |
|---|---|---|---|
| Stadium | 0 – 1 | 0 – 3 | 2 |
| Krofrom | 1 – 4 | 1 – 4 | 3 |
| Tech Junction | 0 – 1 | 1 – 2 | 1 |
| Dichemso | 1 – 2 | 0 – 1 | 2 |

n for lettuce samples =4 for Stadium, 6 for Krofrom, 3 for 'Tech Junction' and 2 for Dichemso

## 4.5 HEALTH RISK ASSESSMENT

### 4.5.1 Production Site

Farmers from all the three selected sites were males with most of them living close to their farms. Each of the studied vegetable production farms had an average of fifteen farmers who were all producing mainly for sale.

During informal Focus Group Discussions, all the farmers admitted having experienced some health problems in the last one year. Some attributed this to their exposure to wastewater. Although most of the farmers acknowledged the risk posed by wastewater use in irrigation, 80% also indicated their health problems could also be due to pesticides and poultry manure use. Farmers mostly reported of malaria (95%), skin and foot rot diseases (50%); diarrhoea (10%) with other complaints being back ache. Although some farmers had family members with similar health problems, they did not attribute this to the consumption of their produce or exposure to wastewater.

During sampling, observed unhygienic practices on the farms included the disposal of waste in irrigation water channels, farmers walking indiscriminately through the irrigation water channels without any Personal Protective Equipment (PPE), washing of their body parts in the dug-wells after the day's work (Plate 4.3).

**Plate 4.3: Farmer working without Personal Protective Equipment**

### 4.5.2 Market Sites

All sellers interviewed at the markets were females and retailers of lettuce and other exotic vegetables. Sixty three percent (63%) of the sellers displayed their produce in receptacles with water for refreshing the produce from time to time to keep them fresh.

Although most of the sellers did not associate any health risk with their produce they admitted the source of irrigation water and the pesticides used could lead to cholera and other diarrhoeal diseases. Some of the possible causes of contamination on the markets could be the practice of washing different types of vegetables in the same bowl of water and the use of refreshing water for other activities as washing of hands before and after eating. The surrounding vegetable market environment on the markets was unclean.

### 4.5.3 Street Food Vendor Sites

Sixty-three percent of the street food vendors were between the ages of 21-30 years, 79% were females and 21% males. Seventy-nine percent had either primary or secondary education, and twenty-one percent had no formal education.

In getting rid of soil particles on their produce, 96% of the sellers washed/cleaned their lettuce with pipe borne water and 4% with well water. 49% of the sellers added salt and 13% added vinegar to the washing water because it could kill pathogens present on the produce. Others simply do that to impact flavour (which some of their customers like) to the lettuce. But most of them prefer salt because claimed it is cheaper and easy to get (Fig. 4.8).

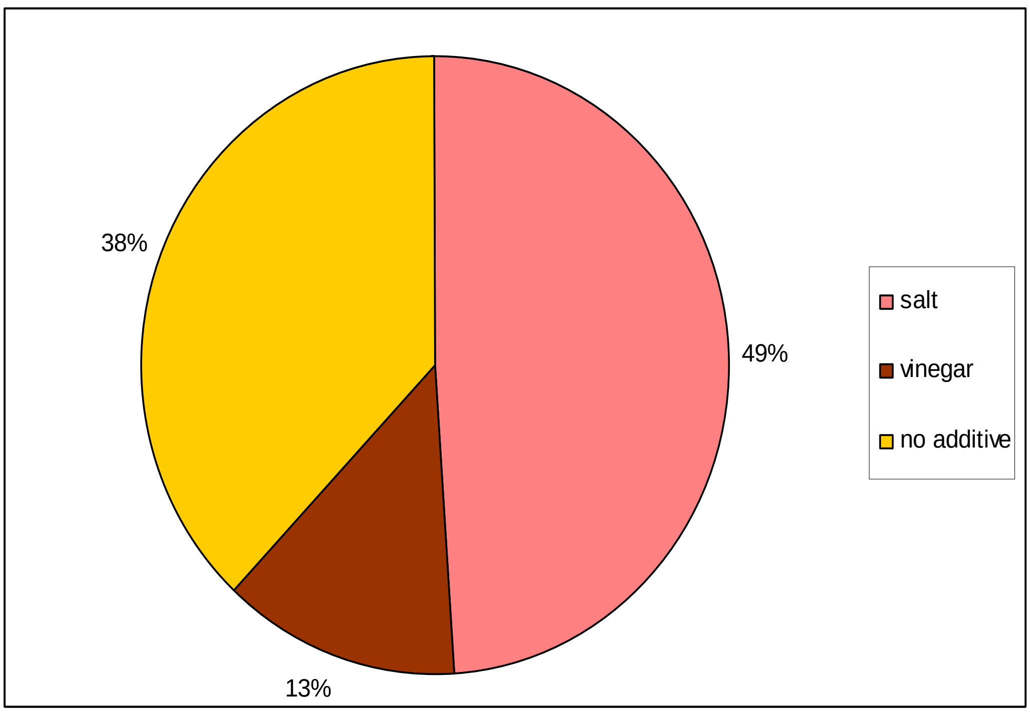



**Figure 4.8:** Types of sanitizing solutions used by street food vendors for washing lettuce

# CHAPTER FIVE

## 5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

## 5.1 DISCUSSION

### 5.1.1 Contamination of Irrigation Water and Lettuce at Production Sites

The study shows that lettuce leaves produced, sold and consumed (from "farm to fork") in urban and peri-urban Kumasi were contaminated with helminths eggs; with the helminths loading decreasing from 4 to 14 eggs $100g^{-1}$ wet weight on farm to 2 to 15 on market and 1 to 3, on the chopped lettuce leaves sold by street food vendors (Tables, 4.2, 4.3 and 4.4). Helminth eggs numbers on farms sampled in this study were much higher compared to work by Ozlem and Sener, (2005) who reported between 1 to 3 eggs on farms studied in Turkey. The difference could be due to the quality of the irrigation water being used by the vegetable and fruit farmers in Turkey.

In our study however, the quality of the irrigation water on the three farm sites were low recording between 3 and 25 helminths eggs/l, exceeding by far the recommended level of <1 egg $l^{-1}$ for unrestricted irrigation (WHO, 1989) and which could account for the higher numbers on the leaves. Amoah *et al.* (2005) recorded between 2 to 4 eggs $l^{-1}$ in some irrigation water sources in Kumasi and Accra while Cornish *et al.* (1999) earlier reported between 1 and 5 helminths eggs $l^{-1}$ in both urban and peri-urban irrigation water sources including shallow wells. In Metropolitan Kumasi, Ghana, irrigation water sources are mainly wastewater from domestic sources that are channelled into water holes; shallow hand dug wells and urban streams which have been shown to be contaminated from diffuse sources (Cornish *et al*, 1999; Keraita *et al*, 2003; Obiri-Danso *et al*., 2005). Badu's farm that uses shallow hand dug wells for irrigation had higher (14 eggs $100g^{-1}$ wet weight) helminths eggs on their lettuce leaves compared to Deduako (3 eggs $100g^{-1}$ wet weight) where water from streams and rivers is used for irrigation.

The higher population of helminths eggs in irrigation water from the shallow/dugout wells is debatable as they are thought to be of good quality due to its natural filtering ability. However, most of the wells on the study farms were not protected from channels purposely created to receive domestic waste from the surrounding residential homes. Additionally, livestock and humans including the farmers often defecate in bushes around their farms due to the unavailability of sanitation amenities. The location of poultry manure heaps also allows for possible runoff into the irrigation water (Drechsel *et al.,* 2000; Amoah *et al*., 2005).

*Ascaris lumbricoides* were the most dominant of the varied helminths eggs detected at all the stages of the production-consumer chain. The other eggs were species of *Shistosoma, Hookworm, Trichuris trichura, Taenia, Clonorchis* and Strongyloides larvae (Table 4.2). On all the three farm sites, *Ascaris lumbricoides* eggs were high compared to the other helminths eggs and represented 75%, 69% and 89% of the total egg count Karikari, Badu and Deduako farms, respectively. On vegetable farms in Marrakech, Bouhoum *et al.* (1997), showed that *Ascaris* eggs numbers were high (75.6 eggs $l^{-1}$) in the irrigation water and again the most dominant (52%) of the helminths eggs in the irrigation water used by the farmers. Similar to the Marrakech farmers, farmers in Kumasi also practice the overhead irrigation technique which could have influenced the transmission of eggs present in the irrigation water unto the leaf surfaces since the broad leaf surface of lettuce exposes much of its surface area to contamination from splashes of soil during irrigation. Secondly, seventy five percent of the organic manure used by farmers in the management of soil fertility is poultry manure and this has been shown to contain high numbers of helminth eggs/larvae (Westcot, 1997; Drechsel and Kunze, 2002).

### 5.1.2 Contamination of Lettuce at Market Sites

Lettuce samples sold on the vegetable markets in Kumasi were also contaminated with helminths eggs/larvae (2-7eggs $100g^{-1}$ wet weight) but there was a 21.6% reduction compared to numbers on the farms (Table 4.2). Drechsel *et al*., (2000) reports that it is difficult to find any irrigated vegetables (lettuce, spring onions, and cabbage) sold on markets that are not contaminated with helminths eggs. Similarly, on the markets, helminths eggs on lettuce leaves were mainly *Ascaris lumbricoides* (1- 6 eggs $100g^{-1}$ wet weight) (Table 3.2). Ulukanligil *et al.,* (2001) reported similar findings in Turkey where he showed that nearly half of the vegetables including lettuce (irrigated with wastewater) sold on the market were contaminated with *Ascaris*. The survival of *Ascaris* could be attributed to their high level of persistence in diverse environments (Feachem *et al*., 1983).

On the European/French Line and Railway markets in Kumasi, sellers use refreshing water to keep the vegetables fresh from the scorching sun as most of these wares are displayed in open receptacles. This study shows that the refreshing waters on the markets in Kumasi contain helminths eggs (4-10 eggs $l^{-1}$). The contamination could be because sellers usually wash vegetables in the same bowl of water before selling, often with one bucket of water per day, thus making the vegetables dirtier over time. Secondly, the lettuce leaves are often subject to possible cross contamination since different vegetables are washed and displayed in the same receptacle. Thirdly, poor handling and cleaning practices by market women contribute to vegetable contamination at the market. Sellers in Kumasi wash their vegetables with the irrigation water at the farm gate before sending them to the markets (Drechsel *et al*., 2000). This study observed a positive correlation between helminths eggs numbers on the lettuce leaves and the refreshing water. Even in the Asafo market where lettuce leaves are displayed without refreshing water, helminths eggs were still high (1-4 eggs $100g^{-1}$ wet weight) above the recommended limit for raw eaten vegetables.

### 5.1.3 Contamination at Street Food Vendor Sites

Helminths eggs were predictably low (1 to 3 eggs $100g^{-1}$ wet weight) but not absent in the chopped lettuce sold with rice by street food vendors at Stadium, Krofrom, Tech Junction and Dichemso (Table 4.4). *Ascaris lumbricoides* and *Shistosoma haematobium* were the only types of helminths eggs identified (Table 4.4). Although the street food vendors wash their vegetables with “salt to taste” water or diluted vinegar solution, ready to eat lettuce is still contaminated with helminths. The difficulty may be in determining the right concentration of the salt-water or vinegar-water mixture and the quantity of lettuce leaves to be washed. Often times the same volume of salt-water or vinegar-water is used several times maybe due to the ignorance of the vendors.

Generally, helminths eggs decreased by 67% from farm to the street food vendor sites. This could be due to eggs being desiccated from their exposure to unfavourable environmental conditions. Larkin *et al.* (1978) reported that microbial die-off on vegetable surfaces is a phenomenon directly related to the original contamination levels and environmental conditions. Factors such as solar radiation, temperature, humidity and rainfall directly affect the persistence of microorganisms (Larkin *et al.,* 1978; WHO, 2002). In spite of this reduction; numbers still exceeded the acceptable recommended level by the WHO.

### 5.1.4 Health Risk

Farmers from the selected farm sites had good knowledge of the health hazards associated with wastewater use in vegetable farming, but could not afford the cost of potable water for irrigation. The wastewater was readily available and some farmers were of the opinion that it contains nutrients (Khouri *et al.,* 1994). WHO (1989) and Cifuentes (1993) have shown that there is a risk of infection for people exposed to wastewater and it is highest for round worms like *Ascaris lumbricoides*, *Trichuris trichura* and Hookworms. The major concern is the evidence that all excreted pathogens can survive in the soil long enough to pose potential risk to farm workers. The incidence of diarrhoea, stomach ache and body itch contracted by the farmers could be associated with their direct contact with water re-used in vegetable farming and to the lack of personal hygiene practices like washing of hands with clean water and detergents on the farm.

It was also observed that farmers did not use any Personal Protective Equipment on the farms (Plate 4.3). They claimed not to feel comfortable in 'Wellington' boots and hand gloves as these slowed down work on the farm. Consumers of these vegetables are at risk since helminth egg/larvae numbers detected in irrigation and refreshing water and on lettuce produced and consumed within urban and peri-urban Kumasi, exceed by far the WHO recommended standard for unrestricted irrigation for vegetables that are likely to be eaten raw ( WHO, 1989). Secondly, the quality of the water used in most of the wash methods employed before consumption can not be guaranteed as being efficient.

## 5.2 CONCLUSION

This study has shown that irrigation water, which are mainly wastewater from domestic sources that are channelled into water holes; shallow hand dug wells and urban rivers and streams, used in vegetable farming in urban/peri-urban Kumasi are contaminated with helminth eggs and the numbers are far above the WHO recommended level. Chopped lettuce served at street food vendor sites are also contaminated with only *Ascaris lumbricoides* and *Shistosoma* eggs. The numbers of eggs/larvae on lettuce leaves were higher compared to what is contained in irrigation water on the farms. This trend was also observed for market lettuce leave samples and refreshing water. Although the contamination of lettuce leaves with helminth eggs/larvae decreases along the production-consumer chain, numbers the numbers were still higher than the WHO recommended level thus exposing farmers as well as consumers to the risk of infections.

Since lettuce contamination takes place at both production (Farm) and distribution (market) sites, appropriate intervention strategies to reduce helminth numbers to acceptable limits will be the provision of proper education on farm practices, post harvest handling and washing methods at both market and street food vendor sites and improved hygienic practices at consumer level.

## 5.3 RECOMMENDATIONS

There is growing awareness that good quality irrigation water is important in the production of safe vegetables and as population increases with demand on limited water resources, information on the best way to utilize the declining water resources while maintaining food safety is important.

It is therefore recommended that:

1. Simple and inexpensive methods of improving the microbial quality of irrigation water at the farm level be researched and developed or an alternative source of water be provided for both irrigation and washing of vegetables at the farm gate.
2. Adoption of safer irrigation methods such as drip or surface irrigation to minimize contact of crops with contaminants present in irrigation water.
3. Farmers should be encouraged to use Personal Protective Equipment during farm work to reduce the risk of infection.
4. Education on the right methods for vegetable washing especially at the point of consumption is increased.
5. Further studies are done on the quantitative assessment of the risk of disease from pathogens present in water used to irrigate crops and epidemiological studies be carried out in the areas of wastewater irrigation to determine the presence of helminth eggs/larvae in order to establish a proper correlation between the egg load in the water and the diseases they are likely to cause to farmers, crop handlers and consumers.

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# APPENDIX 1

## ANOVA AND CORRELATION RESULTS FOR VARIATIONS IN HELMINTH EGG NUMBERS IN IRRIGATION WATER AND LETTUCE ON FARM

**Descriptives**

| | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| total egg count or lettuce leaves | Karikari | 54 | 5.5000 | 4.58772 | .62431 | 4.2478 | 6.7522 | 1.00 | 23.00 |
| | Badu | 54 | 14.6667 | 10.65939 | 1.45056 | 11.7572 | 17.5761 | 3.00 | 65.00 |
| | Deduako | 54 | 4.9074 | 3.32667 | .45270 | 3.9994 | 5.8154 | 1.00 | 22.00 |
| | Total | 162 | 8.3580 | 8.24969 | .64816 | 7.0780 | 9.6380 | 1.00 | 65.00 |
| total egg count in Irrigation Water | Karikari | 54 | 2.6667 | 1.78040 | .24228 | 2.1807 | 3.1526 | 1.00 | 7.00 |
| | Badu | 54 | 24.6667 | 11.56768 | 1.57416 | 21.5093 | 27.8240 | 9.00 | 45.00 |
| | Deduako | 54 | 3.0000 | .82416 | .11215 | 2.7750 | 3.2250 | 2.00 | 4.00 |
| | Total | 162 | 10.1111 | 12.32580 | .96841 | 8.1987 | 12.0235 | 1.00 | 45.00 |

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| total egg count on lettuce leaves | Between Groups | 3233.198 | 2 | 1616.599 | 33.278 | .000 |
| | Within Groups | 7724.037 | 159 | 48.579 | | |
| | Total | 10957.235 | 161 | | | |
| total egg count in Irrigation Water | Between Groups | 17164.000 | 2 | 8582.000 | 187.025 | .000 |
| | Within Groups | 7296.000 | 159 | 45.887 | | |
| | Total | 24460.000 | 161 | | | |

## Karikari and Badu Farms - Irrigation Water

**Descriptives**

EGG_W

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Karikari | 54 | 2.6667 | 1.78040 | .24228 | 2.1807 | 3.1526 | 1.00 | 7.00 |
| Badu | 54 | 24.6667 | 11.56768 | 1.57416 | 21.5093 | 27.8240 | 9.00 | 45.00 |
| Total | 108 | 13.6667 | 13.78337 | 1.32631 | 11.0374 | 16.2959 | 1.00 | 45.00 |

**ANOVA**

EGG_W

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 13068.000 | 1 | 13068.000 | 190.800 | .000 |
| Within Groups | 7260.000 | 106 | 68.491 | | |
| Total | 20328.000 | 107 | | | |

## Karikari and Deduako Farms - Irrigation Water

**Descriptives**

EGG_W

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Karikari | 54 | 2.6667 | 1.78040 | .24228 | 2.1807 | 3.1526 | 1.00 | 7.00 |
| Deduako | 54 | 3.0000 | .82416 | .11215 | 2.7750 | 3.2250 | 2.00 | 4.00 |
| Total | 108 | 2.8333 | 1.39089 | .13384 | 2.5680 | 3.0987 | 1.00 | 7.00 |

**ANOVA**

EGG_W

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 3.000 | 1 | 3.000 | 1.559 | .215 |
| Within Groups | 204.000 | 106 | 1.925 | | |
| Total | 207.000 | 107 | | | |

## Badu and Deduako Farms - Irrigation Water

**Descriptives**

EGG_W

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Badu | 54 | 24.6667 | 11.56768 | 1.57416 | 21.5093 | 27.8240 | 9.00 | 45.00 |
| Deduako | 54 | 3.0000 | .82416 | .11215 | 2.7750 | 3.2250 | 2.00 | 4.00 |
| Total | 108 | 13.8333 | 13.60422 | 1.30907 | 11.2383 | 16.4284 | 2.00 | 45.00 |

**ANOVA**

EGG_W

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 12675.000 | 1 | 12675.000 | 188.489 | .000 |
| Within Groups | 7128.000 | 106 | 67.245 | | |
| Total | 19803.000 | 107 | | | |

## Karikari and Badu Farms - Lettuce Samples

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Karikari | 54 | 5.5000 | 4.58772 | .62431 | 4.2478 | 6.7522 | 1.00 | 23.00 |
| Badu | 54 | 14.6667 | 10.65939 | 1.45056 | 11.7572 | 17.5761 | 3.00 | 65.00 |
| Total | 108 | 10.0833 | 9.37597 | .90220 | 8.2948 | 11.8718 | 1.00 | 65.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2268.750 | 1 | 2268.750 | 33.694 | .000 |
| Within Groups | 7137.500 | 106 | 67.335 | | |
| Total | 9406.250 | 107 | | | |

## Karikari and Deduako Farms – Lettuce

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Karikari | 54 | 5.5000 | 4.58772 | .62431 | 4.2478 | 6.7522 | 1.00 | 23.00 |
| Deduako | 54 | 4.9074 | 3.32667 | .45270 | 3.9994 | 5.8154 | 1.00 | 22.00 |
| Total | 108 | 5.2037 | 3.99944 | .38485 | 4.4408 | 5.9666 | 1.00 | 23.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 9.481 | 1 | 9.481 | .590 | .444 |
| Within Groups | 1702.037 | 106 | 16.057 | | |
| Total | 1711.519 | 107 | | | |

## Badu and Deduako Farms – Lettuce

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Badu | 54 | 14.6667 | 10.65939 | 1.45056 | 11.7572 | 17.5761 | 3.00 | 65.00 |
| Deduako | 54 | 4.9074 | 3.32667 | .45270 | 3.9994 | 5.8154 | 1.00 | 22.00 |
| Total | 108 | 9.7870 | 9.26258 | .89129 | 8.0202 | 11.5539 | 1.00 | 65.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2571.565 | 1 | 2571.565 | 41.248 | .000 |
| Within Groups | 6608.537 | 106 | 62.345 | | |
| Total | 9180.102 | 107 | | | |

# CORRELATIONS

## Kakari farms

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| total egg count on lettuce leaves | 5.5000 | 4.58772 | 54 |
| total egg count in irrigation water | 2.6667 | 1.78040 | 54 |

**Correlations**

| | | total egg count on lettuce leaves | total egg count in irrigation water |
|---|---|---|---|
| total egg count on lettuce leaves | Pearson Correlation | 1 | .437** |
| | Sig. (2-tailed) | . | .001 |
| | N | 54 | 54 |
| total egg count in irrigation water | Pearson Correlation | .437** | 1 |
| | Sig. (2-tailed) | .001 | . |
| | N | 54 | 54 |

**. Correlation is significant at the 0.01 level (2-tailed).

## Badu's Farm

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| total egg count on lettuce leaves | 14.6667 | 10.65939 | 54 |
| total egg count in Irrigation Water | 24.6667 | 11.56768 | 54 |

**Correlations**

| | | total egg count on lettuce leaves | total egg count in Irrigation Water |
|---|---|---|---|
| total egg count on lettuce leaves | Pearson Correlation | 1 | .268 |
| | Sig. (2-tailed) | . | .050 |
| | N | 54 | 54 |
| total egg count in Irrigation Water | Pearson Correlation | .268 | 1 |
| | Sig. (2-tailed) | .050 | . |
| | N | 54 | 54 |

## Deduako Farm

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| total egg count on lettuce leaves | 4.9074 | 3.32667 | 54 |
| total egg count in Irrigation Water | 3.0000 | .82416 | 54 |

**Correlations**

| | | total egg count on lettuce leaves | total egg count in Irrigation Water |
|---|---|---|---|
| total egg count on lettuce leaves | Pearson Correlation | 1 | .048 |
| | Sig. (2-tailed) | . | .729 |
| | N | 54 | 54 |
| total egg count in Irrigation Water | Pearson Correlation | .048 | 1 |
| | Sig. (2-tailed) | .729 | . |
| | N | 54 | 54 |

# APPENDIX 2

## ANOVA AND CORRELATION RESULTS FOR VARIATIONS IN HELMINTH EGG NUMBERS IN REFRESHING WATER AND LETTUCE ON THE MARKET

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Railway | 27 | 6.8519 | 3.32478 | .63985 | 5.5366 | 8.1671 | 2.00 | 18.00 |
| European | 36 | 7.2500 | 3.54864 | .59144 | 6.0493 | 8.4507 | 2.00 | 18.00 |
| Asafo | 27 | 2.0370 | 1.28547 | .24739 | 1.5285 | 2.5456 | 1.00 | 5.00 |
| Total | 90 | 5.5667 | 3.75380 | .39569 | 4.7804 | 6.3529 | 1.00 | 18.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 482.980 | 2 | 241.490 | 27.246 | .000 |
| Within Groups | 771.120 | 87 | 8.863 | | |
| Total | 1254.100 | 89 | | | |

## Railway and European Markets - Lettuce

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Railway | 27 | 6.8519 | 3.32478 | .63985 | 5.5366 | 8.1671 | 2.00 | 18.00 |
| European | 36 | 7.2500 | 3.54864 | .59144 | 6.0493 | 8.4507 | 2.00 | 18.00 |
| Total | 63 | 7.0794 | 3.43277 | .43249 | 6.2148 | 7.9439 | 2.00 | 18.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2.446 | 1 | 2.446 | .205 | .652 |
| Within Groups | 728.157 | 61 | 11.937 | | |
| Total | 730.603 | 62 | | | |

## Railway and Asafo Markets - Lettuce

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Railway | 27 | 6.8519 | 3.32478 | .63985 | 5.5366 | 8.1671 | 2.00 | 18.00 |
| Asafo | 27 | 2.0370 | 1.28547 | .24739 | 1.5285 | 2.5456 | 1.00 | 5.00 |
| Total | 54 | 4.4444 | 3.48402 | .47411 | 3.4935 | 5.3954 | 1.00 | 18.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 312.963 | 1 | 312.963 | 49.260 | .000 |
| Within Groups | 330.370 | 52 | 6.353 | | |
| Total | 643.333 | 53 | | | |

## European and Asafo Markets - Lettuce

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| European | 36 | 7.2500 | 3.54864 | .59144 | 6.0493 | 8.4507 | 2.00 | 18.00 |
| Asafo | 27 | 2.0370 | 1.28547 | .24739 | 1.5285 | 2.5456 | 1.00 | 5.00 |
| Total | 63 | 5.0159 | 3.81631 | .48081 | 4.0547 | 5.9770 | 1.00 | 18.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 419.271 | 1 | 419.271 | 52.873 | .000 |
| Within Groups | 483.713 | 61 | 7.930 | | |
| Total | 902.984 | 62 | | | |

## Railway and European Markets – Refreshing Water

**Descriptives**

EGG_W

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Railway | 27 | 4.7778 | 2.43900 | .46939 | 3.8129 | 5.7426 | 1.00 | 9.00 |
| European | 36 | 15.8333 | 7.66625 | 1.27771 | 13.2394 | 18.4272 | 5.00 | 31.00 |
| Total | 63 | 11.0952 | 8.12943 | 1.02421 | 9.0479 | 13.1426 | 1.00 | 31.00 |

**ANOVA**

EGG_W

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1885.762 | 1 | 1885.762 | 52.011 | .000 |
| Within Groups | 2211.667 | 61 | 36.257 | | |
| Total | 4097.429 | 62 | | | |

# CORRELATIONS

## Railway Market

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| total egg count on lettuce leaves | 6.8519 | 3.32478 | 27 |
| total egg count in Refreshing Water | 4.7778 | 2.43900 | 27 |

**Correlations**

| | | total egg count on lettuce leaves | total egg count in Refreshing Water |
|---|---|---|---|
| total egg count on lettuce leaves | Pearson Correlation | 1 | .285 |
| | Sig. (2-tailed) | . | .149 |
| | N | 27 | 27 |
| total egg count in Refreshing Water | Pearson Correlation | .285 | 1 |
| | Sig. (2-tailed) | .149 | . |
| | N | 27 | 27 |

## European Market

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| total egg count on lettuce leaves | 7.2500 | 3.54864 | 36 |
| total egg count in Refreshing Water | 15.8333 | 7.66625 | 36 |

**Correlations**

| | | total egg count on lettuce leaves | total egg count in Refreshing Water |
|---|---|---|---|
| total egg count on lettuce leaves | Pearson Correlation | 1 | .211 |
| | Sig. (2-tailed) | . | .218 |
| | N | 36 | 36 |
| total egg count in Refreshing Water | Pearson Correlation | .211 | 1 |
| | Sig. (2-tailed) | .218 | . |
| | N | 36 | 36 |

## APPENDIX 3

## ANOVA RESULTS FOR VARIATIONS IN HELMINTH EGG NUMBERS IN LETTUCE AT THE DIFFERENT STREET FOOD VENDOR SITES

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| stadium | 8 | 1.7500 | 1.48805 | .52610 | .5060 | 2.9940 | .00 | 4.00 |
| Krofrom | 12 | 2.5000 | 2.71360 | .78335 | .7759 | 4.2241 | .00 | 7.00 |
| tech | 6 | 1.0000 | 1.26491 | .51640 | -.3274 | 2.3274 | .00 | 3.00 |
| dichemso | 6 | 1.5000 | 1.04881 | .42817 | .3993 | 2.6007 | .00 | 3.00 |
| Total | 32 | 1.8438 | 1.96927 | .34812 | 1.1338 | 2.5537 | .00 | 7.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 10.219 | 3 | 3.406 | .867 | .470 |
| Within Groups | 110.000 | 28 | 3.929 | | |
| Total | 120.219 | 31 | | | |

## ANOVA AND CORRELATION RESULTS FOR VARIATIONS IN BACTERIAL FROM PRODUCTION SITES (FARM) TO CONSUMER STAGE

**Descriptives**

EGG_L

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Farm Sites | 162 | 8.3580 | 8.24969 | .64816 | 7.0780 | 9.6380 | 1.00 | 65.00 |
| Market Sites | 90 | 5.5667 | 3.75380 | .39569 | 4.7804 | 6.3529 | 1.00 | 18.00 |
| Street Food Sites | 32 | 1.8438 | 1.96927 | .34812 | 1.1338 | 2.5537 | .00 | 7.00 |
| Total | 284 | 6.7394 | 6.94418 | .41206 | 5.9283 | 7.5505 | .00 | 65.00 |

**ANOVA**

EGG_L

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1315.165 | 2 | 657.582 | 14.984 | .000 |
| Within Groups | 12331.553 | 281 | 43.885 | | |
| Total | 13646.718 | 283 | | | |

## APPENDIX 4

## QUESTIONNAIRE AND GUIDE ADMINISTERED TO STREET FOOD VENDORS

Location:……………………………………………. Date:…………………………..

### Section A. Personal Data

1. Age i. <20 years [ ] ii. 21-30 years [ ] iii. 31-40 years [ ] iv. >40 years [ ]

2. Sex i. Male [ ] ii. Female [ ]

3. Marital Status i. Single [ ] ii. Married [ ] iii. Divorced [ ] iv. Widowed [ ]

4. Religion i. Christian [ ] ii. Moslem [ ] iii. Traditional [ ] iv. Other [ ]

5. Educational level i.Primary [ ] ii.Secondary/MSLC [ ] iii.Tertiary [ ] iv. Illiterate [ ]

### Section B. Lettuce Sale

6. How long have you been selling street food? ……………….

7. How do you get your stock of lettuce from the source?

    i. Farm gate [ ] ii. Selling point in the market [ ] iii. Delivered by farmer [ ]

8.a. Do you wash your lettuce before chopping? i. Yes [ ] ii. No [ ]

  b. If Yes from where do you get water for washing?

    i. Standing pipe [ ] ii. Well [ ] iii. Stream [ ] iv. River [ ]

9. a. Do you add anything to the washing water? i. Yes [ ] ii. No [ ]

  b. If Yes what do you add? i. Salt [ ] ii. Vinegar [ ] iii. Lime [ ] iv. Other [ ]

  c. Why?.........................................................................................................

### Observational Guide (For Sampler)

1. The hygienic conditions of the surrounding environment.
2. The display of the lettuce, if there is possible cross contamination.

3. Serving of the lettuce. Whether with bare hands and cleaning of hands in dress/rug.

4. Record any other observations made on each sampling date.