KWAME NKRUMAH UNIVERSITY OF SCIENCEAND TECHNOLOGY,

KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

SOIL TRANSMITTED HELMINTH CONTAMINATION OF LETTUCE IRRIGATED WITH WASTEWATER DURING THE TIME OF HARVEST: A CASE STUDY AT BOADI LETTUCE FARM.

BY

EUNICE DUFIE POKU

DECEMBER 2012

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

SOIL TRANSMITTED HELMINTH CONTAMINATION OF LETTUCE IRRIGATED WITH WASTEWATER DURING THE TIME OF HARVEST: A CASE STUDY AT BOADI LETTUCE FARM.

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

BY

EUNICE DUFIE POKU

DECEMBER 2012

DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University except where due acknowledgement has been made in the text.

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

NAME: EUNICE DUFIE POKU
(STUDENT)

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

NAME: Dr. JOHN A. LARBI
(SUPERVISOR)

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

NAME: Rev. STEPHEN AKYEAMPONG
(HEAD OF DEPARTMENT)

DEDICATION

I dedicate this work to my dear mother, Mad. Salome Boateng

ACKNOWLEDGEMENTS

I am most grateful to Almighty God for His strength and protection throughout my entire study. I am also indebted to my supervisor, Dr. J. A. Larbi for his great and invaluable assistance, patience, sacrifice and directions throughout this study. To all the Lecturers of the Departments of Theoretical and Applied Biology and Environmental Science I say a big thank you. I am also grateful to Yaw Owusu Afriyie Kusi, Dennis Amoah and Godwin Quayson for their time and input in making this work a success.

My deepest, appreciation also goes to my parents; Mr. Kwaku Addai-Poku and Madam Salome Boateng for their unflinching support throughout my studies. My acknowledgement would be incomplete if the likes of my siblings; Mr. Collins Osei-Owusu and Mrs. Charity Amponsaa Bediako-Poku are not recognized. To all individuals who helped in diverse ways in making this work a success, I am most grateful.

ABSTRACT

There has been an increasing interest in reuse of wastewater in agriculture over the last few decades due to increased demand for fresh water. Population growth, increased per capita use of water, the demands of industry and of the agricultural sector all put pressure on water resources. However, its use especially if untreated may pose danger to farmers as well as consumers because it may contain pathogens including helminthes. This study investigated soil-transmitted helminth contamination of lettuce cultivated with wastewater during the time of harvest at the Boadi lettuce farm, Kumasi.

A total of 288 lettuce samples and water samples of irrigation wastewater were collected from February and April for examination. Helminth eggs were enumerated using the modified US-EPA concentration method. Focus group discussions were used to assess the risk factors of infection at the harvesting stage.

The major type of helminths encountered were *Ascaris lumbricoides*, Hookworm and *Trichuris trichiura* with *Ascaris lumbricoides* being the most dominant (42.35%) and *Trichuris trichiura* (22.89%) being the least encountered. The total mean helminth eggs for the farm were 3.9 eggs/100g of lettuce. The *Ascaris lumbricoides* eggs ranged from 0-7 eggs per 100g lettuce. Mean helminth egg populations on the lettuce showed a monthly incidence of 3.5/100g, 3.5/100g and 4.5/100g of lettuce for the month of February, March and April respectively. The mean helminth egg populations for the water were 1.5/L, 1.2/L and 1.8/L for the month of February, March and April respectively.

The study indicates that contamination of lettuce with helminths is still a major problem thus e effective farm interventions and washing of vegetables with sanitizers is required to reduce the risk of transmission and infection.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.1 Background

An important ingredient of a healthy diet is raw (fresh) vegetables. Vegetables can become contaminated with enteric bacterial, viral and parasitic pathogens throughout the process of planting to consumption. The extent of contamination depends on several factors that include the use of untreated wastewater and water supplies contaminated with sewage for irrigation, post-harvest handling, and hygienic conditions of preparation in food service or home settings (Amoah, *et al*., 2007; Beuchat, 2002; Simões *et al*., 2000).

Industrial or municipal wastewater is mostly used for the irrigation of crops, mainly in peri-urban communities, due to its easy availability, disposal problems and scarcity of fresh water. Overall, population growth will be the main driving force for a further demand on water resources, and increased wastewater use.

More than 10% of the world's population consumes foods produced by irrigation with wastewater (WHO, 2006).

In many parts of Ghana including Kumasi, wastewater flows from drains into streams, which are usually used for irrigation. Thus wastewater is mostly used in a diluted form mixed with surface runoff and/or stream water (Cornish *et al*., 2001).

Consumers' demand for better quality vegetables is increasing although the perceptions of what is regarded as 'better quality' are subjective. Most consumers consider undamaged, dark green and big leaves as characteristics of good quality leafy vegetables. However, the external morphology of vegetables cannot guarantee safety from contamination.

Fresh vegetables can be agents of transmission of protozoa cysts and helminths eggs and larvae (Choi *et al*., 1982; Coelho, *et al*., 2001; Daryani *et al*., 2008; Erdogrul & Sener, 2005) and outbreaks of intestinal parasitic infections epidemiologically associated with raw vegetables have been reported from developed and developing countries (Ortega *et al*., 1997; Mintz *et al*., 1993).

The sources of contamination extend beyond the use of wastewater on farms. Post-harvest treatment of vegetables including handling, washing, storage, transportation, sorting, packing, cutting and further processing equipment, both poor hygienic and poor personal hygiene practices during food preparation, and/or contact with contaminated soil or fecal matter are all possible sources of contamination. Wild animals may also contribute to the contamination.

The common micro-organisms isolated from vegetable samples include *E. coli, Pseudomonas spp*, *Enterobacter spp*, *Salmonella spp* (Sonou, 2001; Jones and Heaton, 2006). Helminthes and protozoans have also been detected in vegetables collected from the production-consumption chain (Amoah *et al*., 2005).

Effective wastewater treatment can reduce pathogen levels, but in most developing countries it is not an option for the municipal authorities due to the high costs involved (Keraita *et al*., 2002). Most new sewerage treatment plants in Ghana are also operating below their design capacity. As wastewater treatment does not appear to be a realistic option, banning the use of polluted water by urban farmers has also been tried in Accra and other cities. However this has failed because such bans threaten the livelihoods of many individuals, which run contrary to poverty-alleviation strategies.

In these circumstances, urban farmers express significant concerns because their livelihoods are at permanent risk. Any solution to reduce health risks without forcing them to change their (market-driven) cropping patterns or access to water would be appreciated. In addition, Ghana's Tourism Board has started a campaign directed at consumers to promote 'safer vegetables for healthier cities'. This was prompted because tourists were suffering from outbreaks of gastrointestinal disorders after consuming vegetables in urban areas.

Indirect use of wastewater prevails in most developing countries. Indirect use occurs when treated; partially treated or untreated wastewater is discharged to reservoirs, rivers and canals that supply irrigation water to agriculture. Indirect use poses the same health risks as planned wastewater use projects, but may have a greater potential for health problems because the water user is unaware of the wastewater being present. Indirect use is likely to expand rapidly in the future as urban population growth outstrips the financial resources to build adequate treatment works. 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 (WHO, 1989).

Leafy vegetables and herbs include all vegetables and herbs of a leafy nature and of which the leaf (and core) is intended to be consumed raw, e.g. lettuce (all varieties), spinach, cabbages, chicory, leafy herbs (e.g. cilantro, basil, parsley) and watercress (FAO/WHO, 2008). The role of leafy vegetables and herbs in disease outbreak is very important, especially, when they are a component of a salad made up with a dressing and other foods that are equally suitable for transmission of the pathogen.

The water demand for the production of salad vegetables is high and this is often met by use of water obtained directly from natural sources such as streams, rivers, lakes or ponds (FAO/WHO, 2008). Several microbial pathogens can be transmitted to humans through the use of such contaminated water for irrigation farming. Intestinal helminthes; *Ascaris lumbricoides*, *Trichuris trichuria* and parasitic protozoans; *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium parvum* are also found in raw and partially treated waste water (WHO, 2006).

1.2 Justification

Vegetable cultivation is on the increase and people use all sorts of water for the irrigation. One notable observation is the use of wastewater for irrigation. In Kumasi, untreated wastewater is an important source of enteric pathogens to soil as it is used in agricultural irrigation (Strauss, 1985). This presents high risk to farm workers and to consumers of food products irrigated with wastewater. The problem of microbial pollution becomes more serious with these vegetables, because many of them are being consumed raw (Kalavrouziotis *et al.*, 2008). The extent of the pollution increases if the vegetable's edible plant parts are near the ground (Minhas and Samra, 2004).

1.3 Research questions

This study sought to answer the following questions;

• What is the quality of the irrigation water used in lettuce production at Boadi Lettuce farm?

• What is the quality of the lettuce harvested at Boadi Lettuce farm?

1.4 Objective of Study

1.4.1 Main Objective

To determine the soil- transmitted helminth contamination of lettuce irrigated with wastewater during the time of harvest at the Boadi lettuce farm

1.4.2 Specific objectives

These are to assess the;

- Helminthes contamination and coliform counts of irrigation water source used for the lettuce cultivation.
- Presence and concentration of helminths and coliforms of lettuce and
- Examine the potential health implications.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wastewater

Wastewater is any water that has been adversely affected in quality by anthropogenic influence. It comprises liquid waste discharged by domestic residences, commercial properties, industry, and/or agriculture and can encompass a wide range of potential contaminants and concentrations. In the most common usage, it refers to the municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing of wastewater from different sources (www.wikipedia.com).

The main sources of urban wastewater are domestic and industrial. Wastewater mainly comprises water, together with relatively small concentrations of suspended and dissolved organic and inorganic solids. Industrial wastewater is often associated with toxic elements such as heavy metals, but with limited industrial development in most developing countries, the greatest health concern when wastewater is used in agriculture is high levels of pathogenic organisms in untreated wastewater (Keraita *et al.*, 2006). The amounts of heavy metals and synthesized organic compounds generated by industrial activities have increased, and some 10,000 organic compounds are added each year. Many of these compounds are now found in wastewater from most municipalities and communities. As technological changes take place in manufacturing, changes also occur in the compounds discharged and the resulting wastewater characteristics. Wastewater is characterized in terms of physical, chemical and biological composition. Many of the physical, chemical and biological characteristics are interrelated. For example temperature, a physical property, affects both the amount of gases dissolved and the biological activity in the wastewater (Metcalf and Eddy, 2003).

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

2.2 The use of waste water for irrigation

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

The use of polluted water for vegetable farming is more widespread in the more populated cities where safe water is scarce and is used for domestic purposes. From a general survey among open-space farmers carried out in 2002, it was found that about 84% of nearly 800 farmers farming in and close to Accra and almost all 700 farmers in Tamale used polluted water for irrigation, at least during the dry seasons (Keraita and Drechsel, 2002).

Typical urban vegetable farm sizes range from 0.1– 0.2 ha and they increase in size along the urban–rural gradient. As production is market oriented, farming is input and output intensive, particularly in terms of the use of water and such other farm inputs as poultry manure, pesticides and fertilizers. In Ghana, most farmers use watering cans to irrigate (Keraita *et al*., 2003). Large volumes of untreated wastewater end up in urban water bodies, which farmers use for irrigation. Vegetables are the crops most commonly irrigated with polluted water as they are the most demanded cash crops in urban areas (Scott *et al*., 2004; Obuobie *et al*., 2006).

The use of contaminated irrigation water poses health risks to farmers and consumers (Blumenthal *et al*. 2000). In Ghana, water used by urban vegetable farmers has high levels of microbial contamination, and vegetables produced are equally contaminated (Amoah *et al*., 2005). This has been associated with the transmission of diarrhoea in the cities (Mensah *et al*., 2002). One of the factors influencing the microbial quality of farm produce, and thus health risks, is the type of irrigation (Brackett 1999; WHO 2006). Based on the health impacts from wastewater, the WHO has classified irrigation into three distinct categories: flood and furrow, spray and sprinkler, and localised irrigation methods (WHO, 2006).

Flood and furrow irrigation (FI) methods apply water on the surface and pose the highest risks to field workers, especially when protective clothing is not used (Blumenthal *et al*., 2000). Spray and sprinkler are overhead irrigation methods and have the highest potential to transfer pathogens to crop surfaces, as water is applied to edible parts of most crops and because aerosol-borne pathogens are carried further. Localised techniques, such as drip-and-trickle irrigation, present the lowest risk to farmers and cause minimal pathogen transfer to crop surfaces because water is directly applied to the root (Pescod, 1992). Localised irrigation is most expensive and prone to clogging because of the turbidity of polluted water (Martijn & Redwood, 2005). In many developing countries, as a result of rapid urbanisation and the absence of wastewater treatment facilities, urban farmers often use wastewater either directly from sewage drains or indirectly through wastewater-polluted irrigation water. Wastewater use in agriculture is common practice and is increasing as a result of the rising water scarcity worldwide (Scott et al. 2004).

2.3 Potential health risk in using wastewater in Agriculture

In areas where infectious diseases due to enteric pathogens are common, these pathogens are found in very high concentrations in the sewage water. When waste water is used for irrigation without any treatment the pathogens are applied to the agricultural land. This is 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. However, the actual health risk, which is the risk of people falling ill, is lower than the potential health risk (WHO, 1989). The potential health risk is only 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.

Compared with other pathogens, helminths persist for long periods in the environment, host immunity is low to nonexistent and the infective dose is small (Gaspard *et al.*, 1997). Epidemiological studies have shown that the actual risk of infection for people exposed to wastewater is the highest for the roundworm, *Ascaris lumbricoidus,* the whipworm, *Trichuris trichiura* and the hookworms, *Ancylostoma duodenale* and *Necator americanus* (WHO, 1989; Cifuentes 1993, 1994). The risk of acquiring a bacterial, protozoan or fungal infection due to exposure to wastewater is much lower. The survival time of these pathogens in the environment is lower and there is more host immunity. The lowest risk is for viral infections, mainly due to high host immunity for virus infections (WHO, 1989; Swartzbrod, 1995). However, an outbreak of cholera due to consumption of wastewater irrigated vegetables was reported in Israel (WHO, 1989) and a significant higher prevalence of *Entamoeba histolytica* infections and diarrhea in children was observed in a wastewater-irrigated area in Mexico (Cifuentes 1993, 1994).

Although irrigation with wastewater has been practiced for centuries, the first health regulations were developed in the early 20th century. With the growing awareness and fear of transmission of communicable diseases, strict guidelines were set. However, these first health regulations lacked an epidemiological base and were too strict. In 1989, WHO set more realistic guidelines, based on epidemiological evidence (Shuval, 1991). However, recent evaluations show that these guidelines protect crop consumers, but not necessarily field workers and their families, especially children (Blumenthal *et al*., 1996).

2.4 Sources of contamination of vegetables

Conditions and measures taken during pre-harvest and post-harvest affect the microbial contamination of fruits and vegetables.

2.4.1 Pre-harvest

Soil is a rich reservoir for a variety of microorganisms and the non-pathogenic flora is important for the mineralization of plants and animals after their death in the environment. Water is mainly used for the irrigation of plants and its quality varies depending on whether it is surface water or potable water. Water may be a source of contaminating microorganisms and parasites. Surface water from streams and lakes may be contaminated with pathogenic protozoa, bacteria and viruses. The transfer of foodborne pathogenic microorganisms/parasites from irrigation water to fruits and vegetables will depend on the on the irrigation technique and on the nature of the produce (NACMCF, 1999a). Spray irrigation would be expected to increase risk of contamination in comparison to drip irrigation or flooding. Leafy vegetables provide large surfaces for contact with water and for attachment of microbes.

Sewage, manure, slurry, sludge and compost of human and animal origin are commonly used as organic fertilizer for the fruit and vegetables production particularly I organic production systems. The faecal origin of these fertilizers, however, indicates a potential risk of contamination by viruses, bacteria and parasites pathogenic to humans.

2.4.2 Harvest

Fruits and 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 (Buchat, 1995). In an investigation of several foodborne illnesses associated with fresh produce (NACMCF, 1999a); agricultural workers were in many cases the likely source of the pathogen. Clean, well designed and maintained equipment is less likely to cause damage to fresh produce to introduce spoilage and pathogenic microorganisms (Brackett, 19920). Dirty storage facilities and the presence of rodents, birds and insects may increase the risk of contamination with foodborne pathogens. 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

Handling, storage, transportation and cleaning are some of the post-harvest treatment of fruits and vegetables. During these practices, conditions may arise which may lead to cross contamination of the produce from other agricultural materials or from the workers. Environmental conditions and transportation time will also influence the hygienic quality of the produce prior to processing or consumption. Poor handling can damage fresh produce, rendering the product susceptible to the growth/survival of spoilage and pathogenic microorganisms. This damage can also occur during packaging and transport. The presence of cut and damaged surfaces provides an opportunity for contamination and growth of microorganisms and ingress into the plant tissues (Francis *et al*., 1999).

2.5 Pathogens present in Wastewater

Pathogenic microorganisms have been identified to be potentially present in wastewater; these include bacterial, viruses, protozoa and helminths.

2.5.1 Pathogen survival and transport in soil

From the time of excretion by humans the concentration of all pathogens usually goes down as a result of death or loss of infectivity, due to the adverse conditions in the environment outside the human host. The concentration of intestinal nematodes, viruses and protozoa will always decrease, as these can't multiply outside the human host. However, bacteria can multiply if they find themselves in a nutrient rich environment with limited competition from other micro-organisms.

The number of pathogens found in urban sewage will depend on a number of factors which include: the prevalence of a disease in the population discharging into the sewerage systems, the daily per capita water use and the time and distance travelled in the sewerage system. Table 2.1 shows the concentration range of different excreted pathogenic organisms found in urban wastewater.

2.5.2 Bacteria

Pathogenic or potentially pathogenic bacteria are normally absent from a healthy intestine unless infection occurs. When infection occurs, large numbers of pathogenic bacteria will be passed in the faeces thus allowing the spread of infection, with cholera being the worst form. Typhoid, paratyphoid and other Salmonella type diseases are also caused by bacterial pathogens.

Table 2.1: Excreted organism concentrations in urban wastewater

ORGANISM	NUMBERS IN WASTEWATER PER LITRE
Bacteria	
Thermotolerant coliforms	108 – 10^{10}
Campylobacter jejuni	10 – 10^{4}
Salmonela spp.	1 – 10^{5}
Shigella spp.	10 – 10^{4}
Vibrio cholera	102 - 10^{5}
Helminths	
Ascaris lumbricoides	1 – 10^{3}
Hookworms	1 – 10^{3}
Trichuris trichiura	1 – 10^{2}
Schistosoma mansoni	No Data
Protozoa	
Cryptosporidium parvum	1 – 10^{4}
Entamoeba histolytica	1 - 10^{2}
Giardia intestinalis	10^{2} - 10^{5}
Viruses	
Enteric viruses	105 -106
Rotaviruses	102-105

Source: WHO, 2006

2.5.3 Viruses

Numerous viruses may infest humans and are passed in the faeces (>109/g). five groups of pathogenic excreted viruses are particularly important: adenoviruses, enteroviruses (including polioviruses, hepatitis A virus, reoviruses and diarrhea-causing viruses (especially rotavirus).

2.5.4 Protozoa

Many species of protozoa can infect humans and cause diarrhea and dysentery. Infective forms of these protozoa are often passed as cysts in the faeces and humans are infested when they ingest them. Only four species are considered to be pathogenic: *Cryptosporidium parvum, Giardia lamblia, Balantidium coli* and *entamoeba histolytica*. An asymptomatic carrier state is common in all four and may be responsible for continued transmission. *Cryptosporidium* and *Giardia* are the most common Protozoan contaminants of vegetables.

2.5.5 Helminths

Helminth is a term used to describe worms collectively. Worldwide, worms are the principal causative agents of human disease. It is estimated that the number of human infections caused by helminths collectively is on the order of 4.5 billion (Roberts and Janovy, 1996).

2.5.5.1 Classification of helminths

Most of the helminths fall into three phyla: Nematoda (roundworms), Platyhelminths (flatworms), and Annelida (segmented worms). Most human infections are associated with nematodes and flatworms, while the segmented worms such as leaches are primarily ectoparasitic. The phylum collectively represents one of the most abundant animal groups on earth, most of which are harmless to humans. Included among its members are the large roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichuira*), the hook worms (*Necator americanus* and *Ancylostoma duodenale*) and the threadworm (*Strongyloides stercoralis*). *Ascaris lumbricoides* is considered to be the most prevalent parasitic infection worldwide with over one and half billion persons infected (Crompton 1999; Maier et al., 2000; Roberts and Janovy, 1996). It has been estimated that there are on the order of 4 million cases in the United States (Khuroo, 1996).

The phylum Platyhelminthes includes the tapeworm *Taenia saginata* (beef tapeworm) and *Taenia solium* (pork tapeworm) and *Schistosoma* species. *Taenia saginata*, transimitted primarily by infected beef products, is the most common tapeworm found in humans. The trematodes *Schistosoma masoni*, *S.haematobium*, and *S. japonicum*, are also known as blood flukes, are medically important members of trematoda class. More than 200 million infections are ascribed to these worms worldwide.

The human infective stage of helminths varies; in some species it is either the adult organism or larvae, while in other species it is the eggs, but it is primarily the eggs that are present in wastewater.

2.5.5.2 Helminths' life cycle

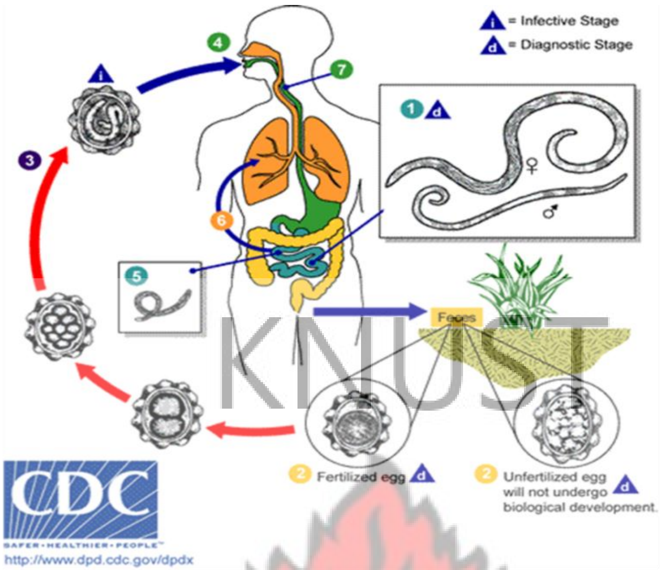
Helminths have different and complex life cycles and ideal living environments. Helminths' life cycle is very different from that of bacteria and protozoan, which are well-known microbes in the sanitary field. The *Ascaris lumbricoide*'s life cycle illustrates these differences well. *Ascaris* eggs are not normally infective and to become so, they need to develop a larva (embryonated egg). The larva develops in the normal temperature and moisture of the external environment (eg. soil and crops) in around 10 days. If a person ingests 1 to 10 *Ascaris* eggs, for instance, the eggs travel to the intestine adhering to the duodenum. There, the larva begins to develop producing an enzyme that dissolves the shell. When the eggs hatch, the larva leaves the egg, crosses the intestine wall and enters the blood stream. Through the blood

Ascaris larva is transported to the heart, lungs and bronchus tubes. The larva remains in the lungs for approximately 10 days before travelling to the trachea from where it is ingested and returned once again to the intestine.

During its journey, many larvae are destroyed, as they are lost in tissues unsuited to their development, but in other cases the larva forms cysts (in the kidneys, bladder, appendix, pancreas or liver) producing damage and requiring surgical removal . Back in the intestine, 2-3 months after its departure, *Ascaris* reaches its adult phase, and, if female, produces up to 27 million eggs. Eggs are passed to the faeces in the unembryonated state and the life cycle begins once again.

Other helminths have an intermediate host, like *Schistosoma* spp. causes schistosomiasis, a common disease in 54 African and some Asian countries. Schistosoma belong to the Trematode group and those infecting humans are colloquially known as blood flukes. During their life cycle schistosomas mature eggs are discharged with faeces into the water. The eggs hatch in response to the temperature and light to release the small free swimming larva miracidia. The miracidia penetrate different classes of fresh snails that serve as intermediate host. In around 4 weeks the miracidia develop via a complex sporocyst scheme to the larva cercarial stage forming a single miracidium and thousands of cercariae are produced. The cercariae are once again excreted to water bodies, infecting humans that come into contact with them by penetrating the skin or by consuming the flesh of polluted fish living in the polluted water (which also serve as hosts). Inside humans, cercariae develop into sexually mature adults migrating to the lungs (in 3-4 days). After penetration of the pulmonary capillaries the worms are carried into the blood stream. In the hepatic circulation schistosomes mature to adults and in pairs they migrate to the mesenteric veins (*S. japonicum* and *S. mansoni*) or to the vesical plexus (*S.*

haematobium). After 35 days (*S. japonicum*, *S. mansoni*) or 70 days (*S. haematobium*) the mature eggs are excreted in faeces and/or urine to begin the cycle once again.



- 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 faeces (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 (6). The larvae mature further in the lungs (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. 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.

- Life cycle image and information courtesy of DPDx

Fig 2.1 Diagrammatic representation of *Ascaris spp* Life cycle

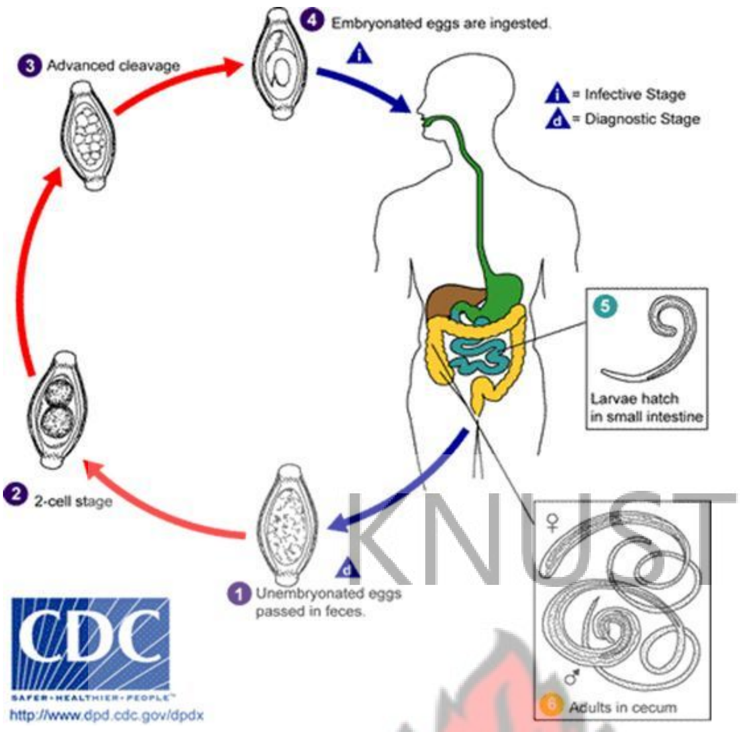


Fig 2.2. Diagrammatic representation of *Trichuris trichiura* life cycle.
(Source: http://www.dpd.cdc.gov/dpdx)

2.5.5.3 Survival of Helminths eggs

Helminths are worms causing a wide variety of diseases globally called helminthiases. Helminthiases almost only occur in developing countries, particularly in areas where sanitation is low. Although helminths are not microscopic animals, their eggs, which are the infective agents, are. Helminth eggs are discharged to the environment in faeces and the oral faecal route is the main dissemination pathway of the disease. The inadequate management and disposal of wastewater, sludge and faecal sludge pollutes crops, water and food that when ingested serve as means of transmitting the disease.

Helminthes eggs which range in size from about 10μm to more than 100μm, can be removed by many commonly used wastewater-treatment processes such as sedimentation, filtration, and stabilization ponds. However, some helminth eggs are extremely resistant to environmental stresses and may survive usual wastewater and sludge disinfection procedures. Chlorine disinfection and mesophilic anerobic digestion, for example, are not effective at inactivating many helminth eggs. In recent study, it has been found that the eggs of Ascaris can survive for up to 10 years in the sediments of oxidation ponds (Nelson, 2001). The long survival times of Ascaris and other worm eggs is a particular importance in the management of bio solids.

An important characteristic of helminth ova is that they have a shell that consists of 3-4 basic layers with a specific chemical composition: a lipoid inner layer, a chitinous middle layer and outer protein layer. All these layers render the eggs very resistant to several environmental conditions. Helminth ova of concern in the sanitary field have a size between 20 to 80 µm and a density of 1.06-1.15 (Ayres *et al*., 1992) and are very sticky. All these properties determine helminth ova's behaviour during treatment. First, it is very difficult to inactivate them unless the temperature is above 40oC or moisture is reduced to below 5% (TS > 95%), according to Feachem *et al*. (1983) and Hays (1977). But details about the contact time under these conditions and other related environmental factors are generally not known. Only contact time at temperatures of around 40oC has been established for one genus of helminth, Ascaris, and, according to US-EPA (1992), it is around 10-20 days. These inactivation conditions cannot be achieved in wastewater treatment but are common in sludge treatment. Thus, helminth ova are removed from wastewater and inactivated in sludge.

2.5.5.4 Sources and routes of transmission

Helminthic parasites can be transmitted to human hosts via food and water. The parasites develop and contaminate food and water during their stages of development. Human hosts are impacted by zoonotic infections or contamination. The lifestyle of the average individual is changing in this age of globalization. People migrating to different areas adopt local consumption habits and lifestyles, and also bring their existing habits from their former homeland. For example, the habits of people from developing countries, consuming raw or undercooked foods, are imported into developed countries. This contributes greatly to the spread of parasitic food-borne infections (Slifko *et al*., 2000).

Many helminths that are transmitted to humans during the consumption of raw mammal meat are cestodes, trematodes and nematodes. Many species of Taenia and Trichinella are also transferred to humans (Pozio 2001). Consuming the raw infected flesh of different animals–from fish to mammals, including slugs and molluscs, and crustaceans–is a source of infection (Hayunga 2001). These parasites infect billions of people around the world, especially in the developing countries. Cestoda are transmitted to humans by the consumption of infected meat or flesh of fish: Trematoda are transmitted to humans by the consumption of raw infected sea and freshwater fish: *Clonorchis sinensis* (freshwater cyprinids), *Opisthorchis* species (freshwater cyprinids) etc.

.Nematode is transmitted to humans via the consumption of infected meat or fish. Raw or inadequately salted, pickled, smoked or cooked fish, molluscs and crustaceans, may be sources of infection, harboring nematode larvae. Human infections are mainly caused by *Capillaria philippinensis* (freshwater fish), *Trichinella* species and other species (pork and other meats). *Capillaria philippinensis* occurs in Taiwan, Korea, Indonesia, India, Colombia, Egypt, and the Philippines; *Trichinella* species are also cosmopolitan, while some species are restricted to some countries; example, *T. nelsoni* is limited to Southeast Africa. Helminths are transmitted to humans through contaminated food, water, and fomites (Van der Hoek *et al*., 2003). The majority are cosmopolitan in distribution, except for ones like Gastrodiscoides and Fasciolopsis, which occur in Southeast Asia, India, European

Russia, the Philippines, and Southeast Asia, Indochina and Japan, respectively; eg, *Fasciola, Fasciolopsis, Dicrocoelium, Gastrodiscoides, Echinococcus, Taenia, Capillaria, Angiostrongylus, Ascaris lumbricoides, Trichuris*, etc. The transmissible stages contaminate food and water directly or indirectly. Infective stages can be passed through fecal matter, reaching the human host by direct consumption or by use of contaminated water during the preparation of food (Lloyd and Soulsby, 1998). The consumption of raw or under-cooked meat and vegetables is the main source of food-borne transmission and risk (Doligalska and Donskow, 2003).

2.5.5.5 Helminthiases

Globally it is estimated that there are almost 1,400 people suffering from helminthiases (WHO, 1996) almost all of them in developing countries. Helminthiases are common in regions where poverty and poor sanitary conditions prevail. Under such circumstances the incident rates may reach 90% (Bratton 1993). There are several kinds of helminthiases named after the helminth causing them. Ascariasis is the most common one and is endemic in Africa, Latin America and the Far East, although the morbidity rate varies according to the region. Almost 73% of *A. lumbricoides* infections occur in Asia, while about 12% occur in Africa and only 8% in Latin American (Peters, 1978) Even though the mortality rate is low; most of the people infected are children under 15 years with problems of faltering growth and/or decreased physical fitness. Children infected with *Ascaris* have proven to be lower in weight and height and have lower haemoglobin concentration and I.Q. than the control group (El- Nofely, 1999). Around 1.5 million of these children will probably never bridge the growth deficit, even if treated. Helminthiases are transmitted through:

(a) The ingestion of polluted crops,

(b) Contact with polluted sludge, faeces or wastewater, and

(c) The ingestion of polluted meat.

Symptoms are different for each helminthiasis but in general they are characterized by haemorrhages, deficient blood coagulation and undernourishment. Helminthiases can degenerate into cancer tumours. During its migration, Ascaris produces allergic reactions (fever, urticaria and asthma). Once back in the intestine, Ascaris produces abdominal pain, meteorism, nausea, vomiting, diarrhea and undernourishment. As mentioned, helminthiases especially affect children. Morbidity rates are higher among the 5-15 year old population and declines markedly in adults. Adults continue to be infected, but their "worm burden" is not as significant, suggesting the development of some level of immunity. Re-infections studies show that when people have been kept free of infection through regular use of anthelmintic drugs, the prevalence of the infections may increase above the pre-treatment value after treatment ceases. In addition, individuals show statistically significant correlations in the numbers of worms harboured after several rounds of treatment (Lorcain, 2000). Therefore, sanitary programmes based only on medical drugs are not enough to control heminthisases; wastewater and sludge treatment also need to be practiced. Additionally, long-term chemotherapy may have negative effects if sanitary conditions are not addressed.

2.5.5.6 Morbidity data

Helminthiases diseases are poorly recorded due to the lack of economical, technical and human resources in places where they are dominant. Data comes mainly from the medical reports of public facilities where helminthiases are identified through the patient's symptoms rather than by using laboratory analysis. Thus, helminthiases are frequently poorly and globally reported (as worm diseases) without indicating the specific type of helminth involved.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted at Boadi, a peri-urban community in the Kumasi metropolis, the capital of the Ashanti region. Kumasi is the second largest city in Ghana and is located between latitude 6^0 42 North and longitude 1^0 35 West and It lies approximately 260m above sea level. Kumasi and is approximately 300 miles (480 km) north of the Equator and 100 miles (160 km) north of the Gulf of Guinea (www.wikipedia.com).

Kumasi features a tropical wet and dry climate, with relatively constant temperatures throughout the course of the year. Kumasi is noticeably wetter than nearby Accra, averaging around 1400 mm of rain per year (Meteorological Services Department, 2002). The city almost features two different rainy seasons, a longer rainy season from March through July and a shorter rainy season from September to November. However, in actuality, the month of March through to November is one long wet season, with a relative lull in precipitation in August. Similar to the rest of West Africa, Kumasi experiences the harmattan during the “low sun” months, lasting from December to February.

The relative Humidity ranges between 1270 to 1410 mm with average daily sunshine durations ranging between 2 to 7 hours and daily minimum temperatures of 21.20^0C and 35.50^0C, respectively (Meteorological Services Department, 2002).

The metropolis has a population of 1,989,06 (Ghana Statistical Service, 2002)and agriculture remains an important livelihood component for many peri-urban residents, with Peri-urban agriculture typically becoming more intensive as the urban area grows and production emphasis shifts towards high value, perishable products such as vegetables which come with a ready urban market.

3.2 Methods and materials

3.2.1 Sampling site

The study was carried out at the Lettuce farm-site at Boadi; a peri-urban community located about 10km from the centre of Kumasi. The farm site has a size of about five hectares and is owned by four farmers with about 21 workers. They obtain their irrigation water from a stream running close to the farm and the irrigation is done by means of watering cans.

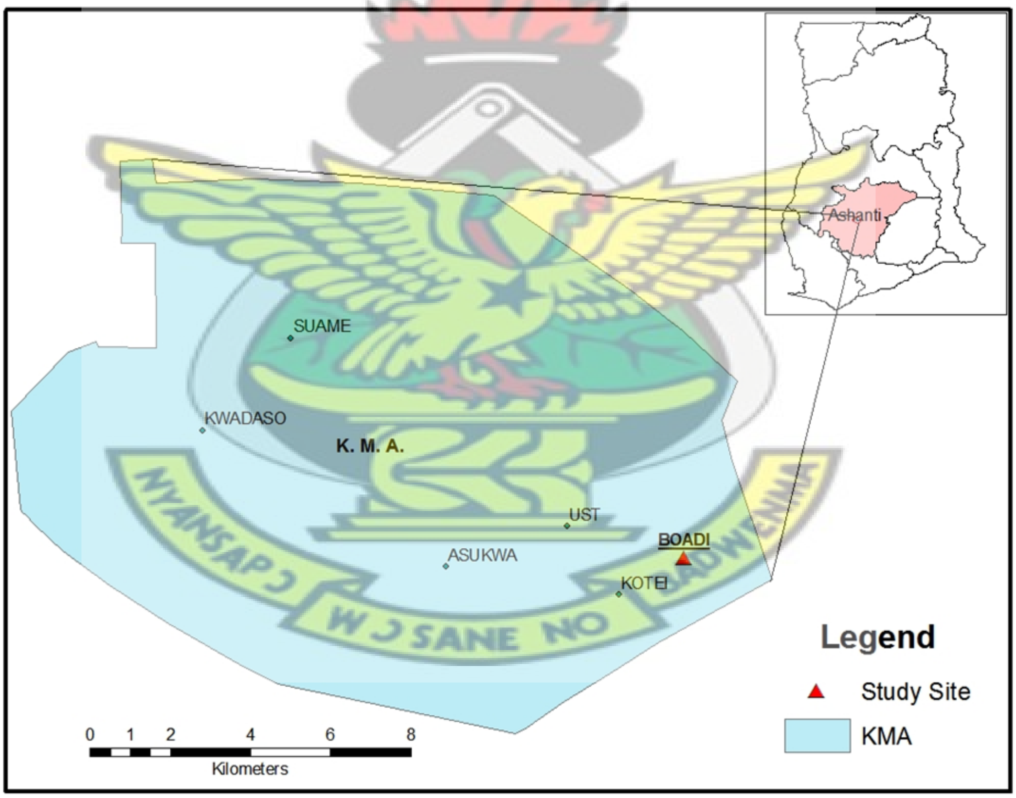


Fig 3.1: Map showing the study location in Kumasi in relation to the rest of Ghana

Plate 3.1. A section of the Boadi lettuce farm

3.2.2 Lettuce sampling

On each sampling date, four lettuce heads per bed were randomly harvested from every third bed in the farm site and placed separately in labeled sterile plastics bags. The samples were then stored in a cool box containing ice packs and transported to the laboratory (Dept. of TAB, KNUST) until processing in the laboratory within 24hours. The lettuce samples were picked in the morning and afternoon.

3.2.3 Irrigation water sampling

Three litres of water samples were taken from the irrigation water source from the farm site on each sampling day into 1.5 litre plastic bottles. The bottles were dipped into the water 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 were then transported to the laboratory in a cool box and analysed within 24 hours. The water samples were collected from the upstream, middle stream and downstream of the course of the stream with reference to the farm.

3.2.4 Preparation of the lettuce and water Samples for enumeration.

Helminth eggs were concentrated for counting using the concentration method outlined by Schwartzbrod (1998). This is a modified US-EPA method, but the same principle of floatation and sedimentation as in the method of Ayres and Mara (1996) was followed. Each of the samples was weighed using a kitchen scale (SOEHNLE, Switzerland) and then washed under running water into a sterilized container. It was then allowed to stand overnight. Two litres of irrigation-water samples were also allowed to settle in a container overnight.

Next day, as much of the supernatant as possible was sucked up using a vacuum pump (Vacuubrand, Germany) and the sediment transferred into eight 50-ml centrifuge tubes. The 2-L containers were rinsed two to three times with tap water and the rinses were distributed into centrifuge tubes. The tubes containing the sediments were then centrifuged at 1500 rpm for 3 min. The supernatant was gently decanted and the deposit was re-suspended in about 150 ml ZnSO4 solution (specific gravity = 1.2). The mixture was homogenized with a sterile spatula and centrifuged again at 1500 rpm for 3 min. The ZnSO4 solution was added to cause the helminth eggs to float leaving other sediments at the bottom of the centrifuge tube. The ZnSO4 supernatant (containing the eggs) was poured back into the 2-L container and diluted with at least 1L of distilled water. This was also allowed to stand for at least 3 hours for the eggs to settle again. As much supernatant as possible was sucked and deposit was then transferred into eight centrifuge tubes. The 2-L container was rinsed two to three times with tap water and the rinsed water added to the centrifuge tubes and centrifuged at 1500 rpm for 3 min. The deposits were regrouped into one centrifuge tube and centrifuged at 1500 rpm for 3 min again. The deposit was re-suspended in 15 ml acid ⁄ alcohol buffer solution (5.16 ml 0.1N H_2SO_4 in 350 ml ethanol) and about 5 ml ethyl acetate was added. The mixture was shaken and the centrifuge tube occasionally opened to let out gas before centrifuging at 2200 rpm for 3 min. After the centrifugation, a diphasic solution (aqueous and lipophilic phase representing the acid ⁄ alcohol and ethyl acetate, respectively) was formed. With a micropipette, as much of the supernatant as possible (starting from the lipophilic and then the aqueous phase) was sucked out leaving about 1 ml of deposit which was then transferred unto a slide and examined under the light microscope.

The number of eggs per litre of water was calculated as follows;

N = AX/PV

Where; **N**= number of eggs per litre. **A**= number of eggs counted on the slide

X=volume of the final product (ml) **P**= volume of the slide (ml)

V= original volume of the sample (L)

3.2.5 Identification of Helminths Eggs

The helminths eggs were identified on the basis of their morphological characteristics- shape and size, and compared with standard eggs on charts (Guerrant, 1995) using 'The Bench Aid for the Diagnosis of Intestinal Parasites' (WHO, 1994) for preliminary identification. The counting was done under a light microscope in both chambers of a haemocytometer at X40 magnification.

3.2.6 Total and Faecal Coliform Analysis

The Most Probable Number (MPN) method was used to determine the Total and Faecal coliform counts on the lettuce and in the water samples. Briefly, serial dilutions of 10-1 to 10-10 were prepared by picking 1ml of the water sample into 9 ml sterile distilled water. One milliliter aliquots from each of the dilutions were inoculated into 5ml of MacConkey Broth with inverted Durham tubes. The tubes were then incubated at 35^{0}C for total coliforms and 44^{0}C for faecal coliforms for 18-24 hours. Tubes showing colour change from purple to yellow with gas collected in the Durham tubes after 24 hours were identified as positive for both total and faecal coliforms. Counts per 100ml were calculated from Most Probably Number (MPN) tables.

3.2.7 Health Risk Assessment

Assessment of health risk practices were evaluated using structured questionnaires, and observation check lists at the farm sites, to document prevailing hygienic practices. The specific health risks examined included irrigation practices, wearing of protective cloths among others.

3.2.8 Data Analysis

Data were analyzed using the SPSS (version 16) software for windows (SPSS Inc, Chicago, IL, USA). The significant differences in mean helminth eggs counts of different samples were analysed using T-Test. Other data analysis, graphs and tables were obtained using the Microsoft® Office ® Excel (Microsoft Corporation, 2003). The statistical analyses were carried out at $P \leq 0.05$ level of significance.

CHAPTER FOUR

RESULTS

A total of 288 lettuce samples were collected from the farm sites out of which 144 lettuce samples were harvested in the morning and the other 144 harvested in the afternoon. Out of the 288 lettuce samples examined, 274 lettuce samples were infected with the helminth eggs which represent 95.1% of the total samples whiles 14 (4.9%) lettuce heads were not infected.

The total mean helminth eggs for the farm were 3.9 eggs/100g of fresh lettuce. The major types of helminths encountered were *Ascaris lumbricoides*, Hookworm and *Trichuris trichiura*. *Ascaris lumbricoides* was the most dominant (42.35%), followed by Hookworm (35.18%) and *Trichuris trichiura* (22.89%). The *Ascaris lumbricoides* egg counts ranged from 0-7 eggs per 100g of fresh lettuce, Hookworm varied from 0-5 eggs per 100g and *Trichuris trichiura* with 0-4 eggs per 100g of fresh lettuce (Table 4.1).

TABLE 4.1: Mean numbers of helminth types on the lettuce samples.

Types of helminth eggs	Mean helminth eggs (per 100g of lettuce)
Ascaris lumbricoides	1.636 ± 1.433
Hookworm	1.360 ± 1.392
Trichuris trichuria	0.884 ± 1.053

4.1 MEAN HELMINTH EGG COUNTS ON FRESH LETTUCE.

Mean helminth egg populations on the lettuce showed a monthly incidence of 3.5eggs/100g of fresh lettuce, 3.5/100g and 4.5/100g for the month of February, March and April respectively, indicating a higher incidence in April, as shown in Table 4.2. There were no statistically significant differences between the counts in February and March (P=0.899) but there were significant difference between February and April (P=0.003) and between March and April (P=0.003).

TABLE 4.2: Mean numbers of helminth eggs on the lettuce leaves in different months.

Month	Helminth eggs per 100g of fresh lettuce
February	3.545 ±2.099
March	3.517 ±1.519
April	4.528 ±2.197

The mean helminth egg numbers on lettuce leaves were 3.010 eggs, 3.425 eggs and 4.237eggs each per 100 grams of fresh lettuce for the morning during the months of February, March and April respectively.

For the afternoon samples, the mean helminth eggs for the February, March and April were 4.079, 3.609 and 4.819 respectively as shown in Fig 4.1.

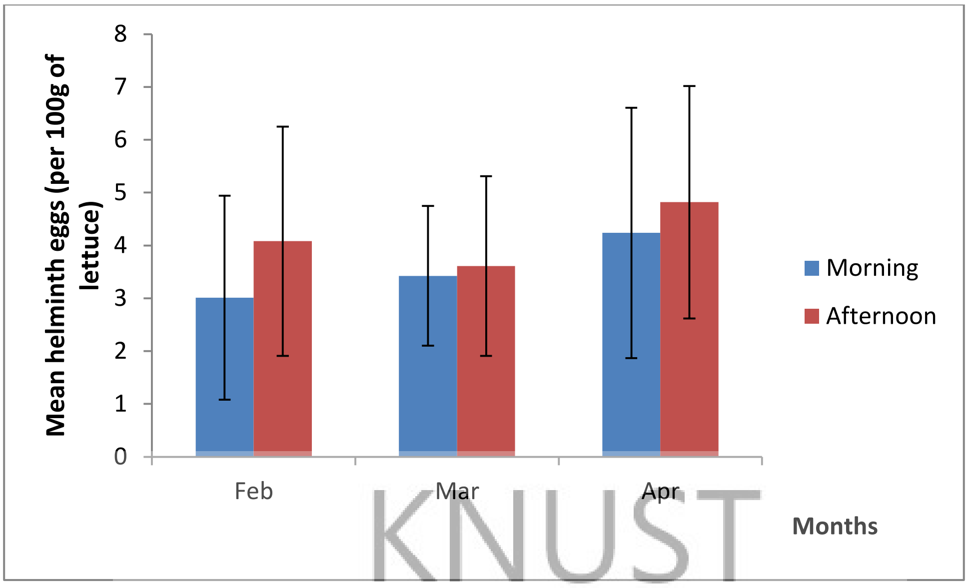


Fig 4.1: Mean numbers of helminth eggs on the lettuce leaves at various times of study.

TABLE 4.3: Mean numbers of helminth eggs on the lettuce leaves in different time of the day for the lettuce samples.

Time of the day	**Mean helmnth eggs (per 100g of lettuce**
Morning	3.557 ± 1.961
Afternoon	4.169 ± 2.013

4.2 MEAN HELMINTHS EGG COUNTS IN THE IRRIGATION WATER USED.

Analysis of the water samples for helminth eggs showed that *Ascaris lumbricoides* had a higher mean incidence of, (3.83 eggs/L) followed by Hookworm (2.75 eggs/L) and *Trichuris trichiura* (2.5 eggs/L) respectively.

4.2.1 Monthly mean Helminth eggs counts.

The mean helminth eggs populations in the water were 1.5/L, 1.2/L and 1.8/L for the month of February, March and April respectively as shown in table 4.3. The main species of helminth eggs found in the irrigation water sample were *Ascaris lumbricoides,* Hookworm and *Trichuris trichiura*. There was no significant difference between the irrigation water between the month of February and March (P=0.235) but there was a difference between the month of March and April (P=0.025).

TABLE 4.4: Monthly Mean numbers of helminth eggs for the irrigation water.

Month	**Mean Helminth eggs (L)**
February	1.500 ± 0.447
March	1.167 ± 0.606
April	1.833 ± 0.516

4.3 COLIFORM LOADS IN IRRIGATION WATER AND ON LETTUCE

The mean Total and Faecal coliform counts of the irrigation water sample was 5.23 X 10^3 cfu/100ml and 5.22 X 10^3 /100ml respectively whereas that for the lettuce were 5.45 X 10^3 cfu/100g for Total coliforms and 5.30 X 10^3 cfu/100g for faecal coliforms (Fig 4.3).

4.3.1 Coliform counts on Lettuce samples

On a monthly basis for the water samples, the month of April showed a higher mean coliform count of 5.31 x 10^3cfu/100mlof total and faecal coliforms than the other two months reported earlier, with mean values of 5.25 x 10^3cfu/100ml and 5.26 x10^3cfu/100mlfor the month of February and March respectively, for the lettuce samples analyzed for both faecal and total coliforms showed the following mean values; 5.26 x10^3cfu/100g, 5.25 x 10^3cfu /100g and 5.32 x 10^3cfu/100g for the months of February, March and April respectively.

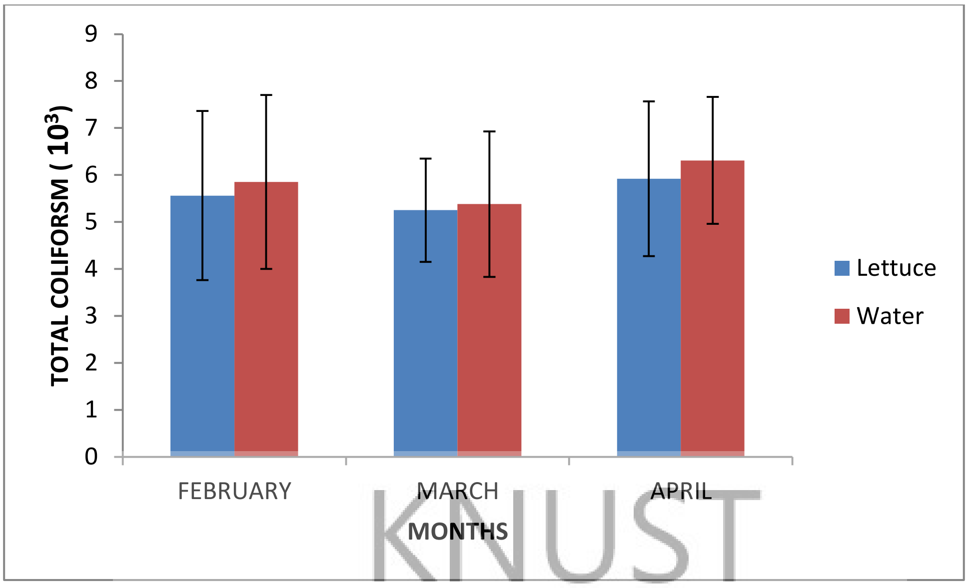


Fig 4.2 Coliform counts at various time of study

4.4 KNOWLEDGE OF HEALTH RISK AMONG FARMERS IN RELATION TO THE USE OF IRRIGATION WATER.

The farmers at the site were both males and females. Some of the farmers have employed young people to help them in the production. In assessing the health risks associated with their work, 80% of the farmers acknowledge that the water they are using for irrigation may pose a negative impact on their health with 20% indicating that their health may be affected by the manure used on the farm.

4.5 FARM PRACTICES

During sampling, some farm practices were observed. These included the disposal of waste in the irrigation channels, none-use of Personal Protective Equipment (PPE) and washing of their body parts in the channels after the day's work. Almost 99% of the farmers had no Personal protective Equipment (PPE) on.

CHAPTER FIVE

DISCUSSION

Vegetable farming using waste water has become a major source of livelihood especially in the urban areas despite the associated health risks. Use of untreated wastewater for crop irrigation causes significant excess infection with intestinal nematodes especially in farm workers and then consumers of their fresh vegetable products, in areas where such infections are endemic.

The study has shown that the lettuce leaves at the time of harvest at the farm site as well as water from the stream which served as the source of irrigation water were contaminated with helminth ova.

5.1 Irrigation water quality.

The main source of irrigation water used by farmers at the study site is a stream which runs through the farms. To protect farmers and consumers from potential adverse health impact of wastewater use in agriculture, WHO has set a helminth guideline of 0 eggs per liter and ≤ 1000 coliforms per 100ml levels in the irrigation water (2006). An average mean count of 1.5eggs/Litre was obtained for the helminth egg counts in the irrigation water. This is above the WHO guideline and indicates that the irrigation water is unacceptable for irrigation of vegetables consumed in the raw state. The faecal coliform contamination level (5.22 X 103.cfu/100ml) in the irrigation water used generally exceeded the recommended standard for unrestricted irrigation. Similar feacal coliform contamination levels in the surface water sources have been reported by Amoah *et al* (2005).

The farm is close to residential areas where most of the houses lack sanitary facilities and domestic livestocks were often found feeding on the farm, thus encouraging resident farmers to defecate in the nearby bushes. The location of the poultry manure heaps also allows for possible runoff into the irrigation water sources and onto vegetable beds (Drechsel *et al*., 2000, Amoah *et al*., 2005).

The main types of helminth eggs isolated in the water *were Ascaris lumbricoides,* Hookworm and *T. trichiuria.*

During the study period, monthly examination of the irrigation water samples gave mean helminth eggs population of 1.5/L, 1.2/L and 1.8/L for the month of February, March and April respectively. There was no statistical difference between the months except between the months of March and April. The high helminth eggs load observed in April could be attributed to the onset of the rainy season. This onset could result in higher runoff rate, thereby leading to an increase in helminth eggs and coliform loads. Anthropogenic activities around the study site may have contributed the presence of the helminth eggs in the irrigation water.

5.2 Quality of the fresh Lettuce leaves

Helminths numbers (per 100g fresh weight) on lettuce ranged between 0-9 eggs. Several factors may have accounted for the levels of helminthes eggs in most of the analyzed lettuce. Among these are the use of polluted irrigation water and also the location of the poultry manure heap which may also allow for the possible runoff into the irrigation water sources and onto the vegetable beds (Drechsel *et al***.,** 2000, Amoah *et al.,* 2005). The water used for irrigation as mentioned above had levels of helminth egg and coliform counts that exceeded the WHO standard, therefore the helminth eggs found on the vegetables might be from the irrigation water used or from the manure and probably from the soil due to splashing during watering or rains.

5.2.1 Lettuce Leaves Quality at the time of Harvest.

The average mean of the helminths eggs population obtained for the months of February, March and April were 1.5/100g, 1.2/100g and 1.8/100g respectively and coliform counts of 5.62 X 10^3, 5.25 X10 and 5.32 X 10^{3} for the months of February, March and April respectively exceeding the WHO limits. The variation of helminth eggs population may be due to the type of activities that occurred within each month including the way irrigation was carried and the rate at which the stream used for irrigation was polluted within each month. As mentioned earlier April is the onset of the rainy season so expectantly the level of helminth loads on the vegetables within this month is expected to have higher levels than the other months. Rainfall causes re-contamination of vegetables through splashes from soils, and wet conditions generally favour pathogen survival (Strauss 1985; Bastos & Mara 1995).

The average mean of helminth eggs obtained for the samples picked in the morning and in the afternoon were 3.6/100g and 4.2/100g respectively. The difference was not statistically significant (P=0.12) Observation made at the farm was that the farms start irrigation of the lettuce after 9:00 in the morning. The morning samples were picked before 9:00 in the morning and the afternoon samples were picked after 12:00 noon. The time of irrigation may have caused the variation between the helminth eggs population in the morning and in the afternoon although they all exceeded the WHO limits.

5.2.2 Types of helminth eggs identified.

The helminth eggs identified were *Ascaris lumbricoides*, Hookworm and *Trichuris trichiuria*. The number of different helminth eggs types isolated from the irrigation water exceeded the WHO (2006) guideline limits of ≤ 1 helminth egg per 100ml for unrestricted irrigation water.

The *Ascaris lumbricoides* was the predominant helminth egg type. This is supported by the results obtained by Amoah *et al.* (2005) who recorded that *Ascaris lumbricoides* formed the greater proportion of helminth eggs isolated on lettuce leaves from Kumasi.

Ascaris lumbricoides are very resistant to the changes in climate, environment and even chemical, living for years in the soil. The eggs are the infective stage to humans when they are ingested through contaminated vegetables or through direct hand to mouth transmission. *Ascaris lumbricoides* can remain viable for several months or years in soil, although often less than two months on crops compared to other helmiths such as Schistosoma species which are likely to survive for more than a couple of days (WHO, 2006).

5.3 Health Risk

Although the farmers at the study site were aware of the fact that the use of wastewater can pose a major setback to their health, they could not have access to potable water for irrigation which is also expensive. Studies carried out by Obuobie (2003) showed that farmers practicing Urban Agriculture in Ghana lack better options hence end up using wastewater sources for irrigation which is in most cases are more reliable and not paid for. Wastewater primarily serves as a source of water but their nutrient has been shown to have fertilizer values and contribute to the improvement of soil properties (Korentajer, 1991). However the benefits of wastewater may be limited by the potential health hazards associated with the transmission of pathogenic organisms from the irrigated soil to crops, grazing animals and humans. The incidence of diarrhoea among farmers was high as it was observed that farmers hardly use personal protective equipment. Furthermore, most of the farmers wash their hands with the irrigation water before eating thus exposing them to infections.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study has shown that, the faecal coliform and helminths contamination level in the irrigation water used for the vegetable farming generally exceeded the recommended standard by WHO for unrestricted irrigation. The month of April had the highest helminths and faecal coliform contamination. The helminth eggs identified were *Ascaris lumbricoides*, Hookworm and *Trichuris trichiuria*. The Ascaris lumbricoides was the predominant helminth egg type. According to the study, the contamination of lettuce with helminths is still a major problem therefore effective farm interventions and washing of vegetables with sanitizers is required to reduce the risk of transmission and infection.

6.2 RECOMMENDATION

The study indicates that the use of wastewater for irrigation of lettuce is the main source of contamination of the lettuce and since it can cause illness by the consuming it raw. It is recommended that;

1. Wastewater used for irrigation should be adequately treated and where possible an alternative source of clean irrigation water should be used.
2. Drip or surface irrigation should be encouraged to minimize the direct contact of crops with wastewater.
3. Farmers should be educated on how to use personal protective equipment to eliminate infection from their exposure to wastewater.
4. Education should be carried out on the need to wash vegetables before consumption.

5. Further studies should be carried out to investigate the quality of manure used on the farm with respect to helminth eggs that are likely to increase the risk of contamination in both the irrigation water used and on the lettuce farm.

REFERENCES

Amoah P, Drechsel P & Abaidoo RC (2005) Irrigated urban vegetable production in Ghana: sources of pathogen contamination and health risk elimination. Irrigation and Drainage 54, S49–S61

Ayres, R., Alabaster, G., Mara, D. and Lee, D. (1992): A Design Equation for Human Intestinal Nematode Egg Removal. In: Waste Stabilization Ponds. Water Research, 26(6): 863-866.

Bastos RKX & Mara DD (1995) .The bacterial quality of salad crops drip and furrow irrigated with waste stabilization pond effluent: an evaluation of WHO guidelines. Water Science and Technology 12, 425–430.

Beuchat, L. R. (2002). Ecological factors influencing survival and growth of human

Blumenthal U & Peasey A (2002) Critical Review of Epidemiological Evidence of the Health Effects of Wastewater and Excreta Use in agriculture. Unpublished document prepared for WHO (available upon request), Geneva.

Blumenthal UJ, Peasey A, Ruiz-Palacios G & Mara DD (2000) Guidelines for wastewater reuse in agriculture and aquaculture: recommended revisions based on new research evidence. WELL study, Task No: 68 Part 1.

Blumenthal, U. J., D. D. Mara, R. M. Ayres, E. Cifuentes, A. Peasey, R. Stott, D. L. Lee and G. Ruiz-Palacios. 1996. Evaluation of the WHO nematode egg guidelines for restricted and unrestricted irrigation. Water Science and Technology 33(10-11):277-283.

Bratton R. and R. Nesse, Postgraduate Medicine, 93, 171 (1993).

Carr R (2001) Excreta-related infections and the role of sanitation in the control of transmission (Chapter 5). In: Water Quality: Guidelines, Standards and Health; Assessment of Risk and Risk Management for Water-related Infectious Disease (L Fewtrell & J Bartram) International water Association (IWA) on behalf on the World Health Organisation, London, UK.

Choi, D. W., Ock, M. S., & Suh, J. W. (1982). Recent demonstration of helminth eggs and larvae from vegetables cultivating soil. The Korean Journal of Parasitology, 20, 83–92.

Cifuentes, E., U. Blumenthal, G. Ruiz-Palacios, S. Bennett and A. Peasey. 1994. Escenario epidemiologico del uso agricola del agua residual: el valle del mezquital, Mexico. Salud Publica de Mexico. 36(1):3-9.

Cifuentes, E., U. Blumenthal, G. Ruiz-Palacios, S. Bennett, M. Quigley, A. Peasey and H. Romero- Alvarez. 1993. Problemas de salud asociados al riego agricola con agua residual en Mexico. Salud Publica de Mexico 35(6):614-619.

Coelho, L. M., Oliveira, S. M., Milman, M. H., Karasawa, K. A., & Santos, Rd. (2001). Detection of transmissible forms of enteroparasites in water and vegetablesconsumed at school in Sorocaba, Sao Paulo state, Brazil. Revista da Sociedade Brasileira de Medicina Tropical, 34, 479–482 [in Portuguese].

Cornish, G.A., Aidoo, J. and Ayamba, I., 2001. Informal Irrigation in the Peri-urban Zone of Kumasi: An Analysis of Farmer's Activity and Productivity. Report OD/TN 103, February 1999, HR Wallingford Ltd, Wallingford, UK, 86 pp.

Crompton, D. W. T. (1999).How much human Helminthias is there in the world? Journalof Parasitology, vol. 85, pp. 379-403.

Daryani, A., Ettehad, G. H., Sharif, M., Ghorbani, L., & Ziaei, H. (2008). Prevalence of intestinal parasites in vegetables consumed in Ardabil. Iran Food Control, 19.790–794.

Drechsel, P., Abaidoo, R.C., Amoah, P., & Cofie, O.O. (2000) Increasing Use of Poultry Manure in and Around Kumasi, Ghana: Is Farmers' Race Consumers' Fate? Urban Agriculture Magazine, 2:25-27

El- Nofely and A. Shaalan, *International Journal of Anthropology*, 14, 55 (1999).

Erdogrul, O. R., & Sener, H. (2005). The contamination of various fruit and vegetablewith Enterobius vermicularis Ascaris eggs, Entamoeba histolytica cysts and Giardia lamblia cysts. Food Control, 16, 557–560.

FAO/WHO 2008.Viruses in Food: Scientific Advice to Support Risk Management Activities. Meeting Report. Microbiological Risk Assessment Series 13. Rome.

Feachem, R., Bradley, D., Garelick, H. and Mara, D. (1983): Sanitation and disease: Health aspects of excreta and wastewater management, John Wiley and Sons, New York, NY.

Gaspard, P., Y. Ambolet, and J. Schwartzbrod. (1997). Valorisation des boues de stations d'épuration envue de l'amélioration des sols destinés à l'agriculture: contamination parasitaire et modélisation en vue dela gestion du risque sanitaire. Bulletin de l' Academie Nationale de Medicine 181(1):43-56.

Hays, B. D. (1977): Potential for parasitic disease transmission with land application of sewage plant effluents and sludge. Water Research, 11: 583-595.

Keraita, B. Danso, G. and Drechsel, P. (2003) Irrigation methods and practices in urban agriculture in Ghana and Togo. Urban Agriculture Magazine; 10: 6–7.

Keraita, B. Drechsel, P. Amoah, P. and Cofie, O. (2006). Assessment of health risks andbenefits associated with UA: impacts assessment, risk mitigation and healthy public policy. Urban Poverty and Environment Series Report #1.IDRCCRDI

Keraita, B., Drechsel, P. and Amoah, P., (2003). Influence of urban wastewater on stream water quality and agriculture in and around Kumasi, Ghana. Environment and Urbanization 15(2), 171–178.

Keraita, B.N. and Drechsel, P. (2002) Agricultural Use of Untreated Urban Wastewater in Ghana. In Wastewater use in irrigated agriculture: Confronting the Livelihood and Environmental Realities. Editors: C.A.Scott, N.I. Faruqui and L. Raschid-Sally.

Khuroo, M.S. (1996) Ascariasis, Gasteroenteriology Clinics of North America, vol. 25, no. 3, pp. 553-577.

Korentajer, L. (1991). A review of the agricultural use of sewage sludge: benefits and potential hazardo.Water Air Soil Pollut., 17: 189-196

Lorcain P. O and C.V. Holland, 121, S51 (2000).

Maier, R.M,.I.L. Pepper, and C.P Gerba (2000) Environmental Microbiology, Academic Press, A Harcourt Science and Technology Company, San Diego, CA.

Martijn E & Redwood M (2005) Wastewater irrigation in developing countries – limitations for farmers to adopt appropriate practices. Irrigation and Drainage 54, S63–S70.

Mensah P, Yeboah-Manu D, Owusu-Darko K & Ablordey A (2002) Street foods in Accra, Ghana: how safe are they? Bulletin of the World Health Organization 80(7), 546–554.

Metcalf & Eddy, Inc., [2003]. Wastewater Engineering Treatment and Reuse.

Minhas, P. S. and Samra, J. S. (2003) Quality assessment of water resources in Indo-Gangetic basin part in India, Central Soil Salinity Research Institute, Karnal, India. p. 68.

Mintz, E. D., Hudson-Wragg, M., Meshar, P., Carter, M. L., & Hadler, J. L. (1993). Foodborne giardiasis in a corporated office setting. *Journal of Infectious Diseases*, 167, 250–253

NACMCF. 1999a. National Advisory Committee on Microbiological Criteria for Foods. Microbiological safety evaluations and recommendations on fresh produce. Food Control 10:117-143.

Nelson, k. (2001) Personal communication, Department of Civil and Environmental Engineering, University of California at Davis, Davis, CA.

Obuobie E., Keraita, B. Danso, G. Amoah, P Raschid, L. and Drechsel, P. (2006). Irrigated Urban Vegetable Production in Ghana Characteristics, Benefits and Risks. IWMI-RAUF-CPWF

Ortega, Y. R., Roxas, C. R., Gilman, R. H., Miller, N. J., Cabrera, L., Taquiri, C., et al. (1997). Isolation of Cryptosporidium parvum and Cyclospora cayetanensis from vegetables collected in markets of an endemic region in Peru. American Journal of Tropical Medicine and Hygiene, 57, 683–686.

pathogens on raw fruits and vegetables.Microbes and Infection, 4, 413–423.

Pescod MB (1992) Wastewater Treatment and Use in Agriculture- FAO Irrigation and Drainage paper 47. FAO, Rome.

Peters W. II. Symposia of the British Society for Parasitology 16, 25 Oxford, Blackwell Scientific Press (1978).

Roberts, L.S and J. Janovy, Jr, (1996) Foundations of Parasitology, 5th ed., WCB, Wm. C. Brown Publishers Dubuque, IA.

Schwartzbrod, L. (1995). Effect of human viruses on public health associated with the use of wastewater and sludge in agriculture and aquaculture. Geneva, Switzerland: World Health Organization.

Scott CA, Faruqui NI & Raschid-Sally L (2004) Wastewater use in irrigated agriculture: management challenges in developing countries. In: Wastewater Use in Irrigated Agriculture: Confronting the Livelihood and Environmental Realities. (eds CA Scott, NI Faruqi & L Raschid-Sally) CABI Publishing, Oxfordshire, UK, pp. 1–10.

Scott CA, Faruqui NI & Raschid-Sally L (Eds) (2004) Wastewater Use in Irrigated Agriculture: Confronting the Livelihood and 3 Environmental Realities. CABI, Wallingford.

Shuval, H. I. 1991. Health guidelines and standards for wastewater reuse in agriculture: historical perspectives. Water Science and Technology 23:2073-2080.

Simões, M., Pisani, B., Marques, E. G. L., Prandi, M. A. G., Martini, M. H., Chiarini, P. F.T., et al. (2001). Hygienic-sanitary conditions of vegetables and water from kitchen gardens in the Municipality of Campinas, SP. Brazilian Journal of Microbiology, 32, 331–333

Slifko, T.R., H.V. Smith and J.B. Rose, (2000). Emerging parasite zoonoses associated with water and food. Int. J. Parasitol., 30: 1379-1393.

Sonou, M., (2001). Periurban irrigated agriculture and health risks in Ghana. Urban Agric. Mag., 3: 33-34.

Strauss M (1985) Health Aspects of Night soil and Sludge Use in Agriculture and Aquaculture – Part II. Pathogen Survival. International Reference Centre for Waste Disposal (now SANDEC), Duebendorf, Switzerland. Report no. 04/85.

US-EPA (1992): Control of pathogens and vector attraction in sewage sludge. EPA/625/R-92-004. Washington, D.C.

WHO (2006) Guidelines for the Safe Use of Wastewater, Excreta and Greywater. Vol. 2 Wastewater Use in Agriculture. WHO, Geneva.

WHO (2006).Guidelines for the safe use of wastewater, excreta and grey water: Wastewater use in agriculture (Volume 2). WHO: Geneva, 219 pp.

WHO (World Health Organization). 1989. Health guidelines for the use of wastewater in agriculture and aquaculture. WHO Technical Report Series 778 Geneva, Switzerland: World Health Organization.

WHO, 2006. Guidelines for the safe use of wastewater, excreta and grey water: Wastewater use in agriculture (Volume 2). Report of a WHO Scientific Group. WHO Technical Report Series 778. World Health Organization, Geneva, Switzerland, pp74-219.

WHO, Fostering Development. World Health Organization, Geneva, 5-7 December WHO/CDS/IPI/96.1. WHO (1996).

WHO. 1989. Health guidelines for the use of wastewater in agriculture and aquaculture: Report of a WHO Scientific Group. WHO Technical Report Series 778. World Health Organization, Geneva. 74 p

APPENDIX

Statistics

		february	march	April
N	Valid	96	96	96
	Missing	48	48	48
Mean		3.5447	3.5170	4.5282
Std. Deviation		2.08865	1.51188	2.18495

T- TEST FOR VARIATIONS BETWEEN THE MONTH OF FEBRUARY AND MARCH IN THE LETTUCE SAMPLES

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
february	96	3.5447	2.08865	.21317

One-Sample Test

	Test Value = 3.517					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper

One-Sample Test

	Test Value = 3.517					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference Lower	Upper
february	.130	95	.897	.02769	-.3955	.4509

T- TEST FOR VARIATIONS BETWEEN THE MONTH OF MARCH AND APRIL IN THE LETTUCE SAMPLES

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
march	96	3.5170	1.51188	.15431

One-Sample Test

	Test Value = 4.528					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference Lower	Upper
march	-6.552	95	.000	-1.01098	-1.3173	-.7046

T- TEST FOR VARIATIONS BETWEEN THE MONTH OF FEBRUARY AND APRIL IN THE LETTUCE SAMPLES

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
April	96	4.5282	2.18495	.22300

One-Sample Test

	Test Value = 3.545					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
April	4.409	95	.000	.98319	.5405	1.4259

T- TEST FOR VARIATIONS BETWEEN THE MORNING AND AFTERNOON LETTUCE SAMPLES

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
afternoon	144	4.1693	2.01339	.23728

One-Sample Test

	Test Value = 3.557					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
afternoon	2.581	71	.012	.61232	.1392	1.0854

T- TEST FOR VARIATIONS BETWEEN THE MARCHWATER AND FEBRUARY WATER SAMPLES

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
MARCHWATER	6	1.1667	.60553	.24721

One-Sample Test

	Test Value = 1.500 (FEBRUARYWATER)					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
MARCHWATER	-1.348	5	.235	-.33333	-.9688	.3021

T- TEST FOR VARIATIONS BETWEEN THE FEBRUARY WATER AND APRILWATER SAMPLES

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
APRILWATER	6	1.8333	.51640	.21082

One-Sample Test

	Test Value = 1.500 (Februarywater)					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
APRILWATER	1.581	5	.175	.33333	-.2086	.8753

T- TEST FOR VARIATIONS BETWEEN THE MARCHWATER AND APRILWATER SAMPLES

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
APRILWATER	6	1.8333	.51640	.21082

One-Sample Test

	Test Value = 1.167 (Marchwater)					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
APRILWATER	3.161	5	.025	.66633	.1244	1.2083