KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

HELMINTH INFECTION RISK ASSOCIATED WITH THE USE OF WASTEWATER IN URBAN AGRICULTURE IN KUMASI, GHANA

A THESIS SUBMITTED 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 PHILOSOPHY (PARASITOLOGY)

BY

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MAY, 2014

DECLARATION

I hereby declare that this thesis presented to the Department of Theoretical and Applied Biology in partial fulfilment for the award of M. Phil. Degree, is a true account of my own work except for the referenes that have been duely acknowledged.

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ABSTRACT

In many cities across developing countries wastewater is largely untreated and ends up being used for urban vegetable farming. The practice has many benefits but can also lead to significant health risks if not undertaken in a safe manner. The main aim of this study was to assess the helminth risk associated with the use of wastewater for irrigation in the Kumasi Metropolitan Area in the Ashanti Region of Ghana. The specific objectives were; a) to assess the occurrence and seasonality of helminth parasite in wastewater used for irrigation and in wastewater contaminated soil. b) to assess the helminths infection risk for urban farmers using wastewater for irrigation; and c) to assess the helminth infection risk for consumers of wastewater irrigated vegetables. Helminth egg concentration was determined using the Modified EPA Protocol. Four types of helminth eggs were identified in both the irrigation water and soil. These were, *Ascaris lumbricoides*, hookworm, *Trichuris trichuira* and *Taenia spp*. *Schistosomaspp* eggs were also found only in irrigation water, with *A. lumbricoides* being the most abundant in the irrigation water and farm soil. There was seasonal variation in the concentration of these helminths, however *A. lumbricoides* had the highest concentration across both seasons. *A. lumbricoides* and hookworm were the only helminth eggs identified in salad foods in the study area. The annual risk of infection with *A. lumbricoides* for vegetable farmers was found to be 0.85×10^{-1}, higher than the tolerable risk of *A. lumbricoides* infection for farmers using wastewater for irrigation (1.2×10^{-2}). Consumers of wastewater irrigated vegetables were equally at risk of *A. lumbricoides* infection with about the same magnitude as the farmers (2.6×10^{-1}).

To reduce the risk of *A. lumbricoides* infection it is recommended that simple, practical and inexpensive interventions such as the practice of drip irrigation, the use of stabilization ponds be introduced on the farms so as to reduce the concentration of these helminths in the irrigation water and soil. It is hope that these interventions would also include measures that reduce the risk of infection to the farmers as well. Education on disinfection practices should be introduced to further reduce the risk of *A. lumbricoides* infection to consumers

DEDICATION

To my father, WO1 Robert Amoah (retired) and my late mother Mrs Regina Amoah

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Extent and drivers of Wastewater use

It is estimated that at least 3.5 to 4 million hectares of agricultural land in 50 countries are irrigated with raw, treated or partially treated wastewater from domestic and industrial sources (Seidu*et al*, 2008). The use of wastewater has been part of human civilizations for centuries, particularly in Far East countries like China. The use of wastewater in agriculture was also widely practiced in some European countries before the advent of advanced treatment technologies (AATSE, 2004). More recently, wastewater use has become an adaptation strategy to combat dwindling freshwater resources available for crop production. This is particularly more evident in water stressed countries in the Middle East and Australia. In Israel, more than 60% of treated wastewater is used for agricultural irrigation (Dreizin, 2011), while in Jordan, 10% of fresh water supply is from reclaimed wastewater (Yasser *et al.,* 2000). In Australia there are several agricultural fields depending largely on treated wastewater (Hamilton *et al*, 2006). Wastewater use in agriculture has also been promoted as part of the concept of sustainable development. Wastewater contains vital plant nutrients including phosphorus, which is a rapidly dwindling global reserve. In many cities in developing countries, the use of wastewater is a common reality experiencing rapid urbanization. UN-Habitat (2004) predicted that by 2030, 60% of the world's population would be living in urban areas and it is projected that the towns and cities of the developing world will make up 80% of the global population (UNFPA, 2007). These changes pose significant implications for urban water and wastewater management. WHO (2000) reports that lack of resources for effective wastewater treatment facilities in most developing countries have contributed to large volumes

of wastewater generated especially in urban areas remaining untreated. This report also estimated that median levels of treated wastewater in Asia would be about 35% and 14% in Latin America and Caribbean, respectively but an abysmal 0% in Sub-Saharan Africa (SSA). Hence, large amounts of this untreated wastewater end up being used by farmers for crop production. The use of wastewater for irrigation is either direct or indirect. The direct use of wastewater is largely in and around large cities in developing countries whereas the indirect use of wastewater involves the discharge of this water into other water bodies such as rivers, streams and canals as irrigation water for farmers. In SSA it is estimated that 10% of the population in some cities is involved in wastewater irrigation. The use of wastewater for urban vegetable production has been reported in Ghana (Cornish and Keilen, 2004), South Africa (Grobicki and Cohen (1999) and Senegal (Gaye and Niang, 2002).

The practice of wastewater irrigation in urban agriculture has been shown to improve the livelihoods of farmers, contribute to the urban food basket and improve the urban environment by diverting wastewater to agricultural fields. In West Africa between 50% and 90% of vegetables consumed by urban dwellers are irrigated with wastewater polluted surface water within or close to cities (Drechsel *et al.*, 2006). In Ghana a significant proportion of wastewater generated is discharged into drains and nearby water bodies untreated which is then used by farmers for irrigation. A survey carried out in 2002 found out that about 54% of nearly 800 farmers in Accra and almost all 700 farmers in Tamale used water for irrigation especially in the dry season. (Klutse, 2009).In Kumasi the second largest city in Ghana, farmers use polluted water sources on about 12,000 hectares. This figure is more than twice the area covered by the formal irrigation schemes in Ghana (Drechsel *et al.*, 2006). About 90% of vegetables consumed in cities is produced through irrigated agriculture, underlying its importance in providing a major

source of income for households (Drechsel *et al*, 2001).Wastewater irrigation in cities is mainly in the production of vegetables including cabbage (*Brassica oleraceacapitata*), spring onion (*Allium fistulosum*), carrots (*Daucus carota spp. sativus*), tomato (*Lycopersicon esculentum*), onion (*Allium cepa*), shallots (*Allium escalonicum*), eggplant (*Solanum melongena*), local spinach (*Amaranthus spp*), Cucumber (*Cucumis sativa*) and lettuce (*Lactuca sativa*) (Andoh, 2006).City consumers are more exposed to multi-cultural diets as compared to rural areas. Often with less time and space for food preparation. There is an increasing demand for convenience and more exotic foods. Although it is difficult to distinguish the contribution of wastewater irrigated agriculture to livelihoods, food chains and nutrition, Amoah (2009) reported that everyday in Accra, more than 200,000 people eat vegetables grown with wastewater.

Although there are many benefits associated with wastewater irrigation as mentioned, the practice can lead to significant health risk if not undertaken in a safe manner (WHO, 2006). All pathogens of viral, bacteria, parasitic and protozoan origins can be found in wastewater; and can be transmitted to farmers using the wastewater for irrigation; consumers of the wastewater irrigated vegetables and populations living in close proximity to areas where the irrigation is taking place.

Faruqui *et al.,* (2004) reported that in Pakistan, farmers using raw wastewater are five times more likely to be infected by hookworms than those using canal water. In Dakar, 60% of the farmers using raw wastewater were infected with either amoebae, which cause amoebic dysentery, roundworms, which cause ascariasis, whipworm, or threadworms (Faruqui *et al.,* 2004). In India, studies have shown that sewage farm workers exposed to raw wastewater in areas where hookworm and *A. lumbricoides* infections were endemic had significantly higher levels of infection than other agricultural workers (Blumenthal, 2000). Research conducted in Phnom Penh, Cambodia indicated an association between exposure to wastewater and skin problems such as

eczema (van der Hoek *et al*., 2005). Although the cause of these skin problems was not determined it is most likely due to a mixture of chemical and biological agents in the wastewater. In a study in Mexico, irrigation with untreated or partially treated wastewater was directly responsible for 80% of all *A. lumbricoides* infections and 30% of diarrheal disease in farm workers and their families (Cifuentes *et al*, 2000).The health risk can also differ according to age and gender. An epidemiological study by Habbari *et al*., (2000) undertaken to determine possible risk associated with raw wastewater use in agriculture in Morocco found ascariasis infection to be approximately five times higher especially in children in wastewater impacted regions compared to control regions.

Therefore there is a need to balance the risk and benefits associated with the practice when developing wastewater irrigation schemes. Underlying this delicate balance is a need to understand the health risks associated with wastewater irrigation to enable the development of effective and cost-effective interventions. This study forms part of a comprehensive study that is assessing the health risk associated with wastewater irrigation in urban Ghana.

From studies conducted in Accra, Kumasi and Tamale, Amoah (2009) reported the presence of total and feacal coliforms in irrigation water used within these cities, in addition, five helminth eggs were isolated with *A. lumbricoides* being the most prevalent, the others were; *Hynmenolepis diminuta, Trichuris trichuira, Fasciola hepatica and Strogyloides* larvae. Similar results were obtained by Cornish *et al.,* (1999) from studies conducted in Kumasi.

1.2. STUDY AIM

To assess the helminth risk associated with the use of wastewater for irrigation.

1.3. Specific objectives

1) To assess the occurrence and seasonality of helminth parasite in wastewater used for irrigation and in wastewater contaminated soil.

2) To assess the helminths infection risk for urban farmers using wastewater for irrigation;

3) To assess the helminth infection risk for consumers of wastewater irrigated vegetables.

1.4. JUSTIFICATION

The use of wastewater for agricultural purposes is a widespread practice in many developing countries of which Ghana is no exception. Most of the vegetables sold in major cities in Ghana especially Kumasi and Accra are produced using wastewater for irrigation. In thelast 10years there has been growing interest in understanding untreated wastewater use and this has lead to the production of large array of information on the importance of this practice. It has also been made abundantly clear that the approach of banning the largely informal practice will not work (Scott *et al*., 2004). Therefore the main challenge is how to maximize the benefits of wastewater use while safeguarding public health and the environment as well. The agricultural use of wastewater comes with several health concerns as mentioned earlier, key among these is the spread of intestinal helminths. *Ascaris* is the most prevalent helminth infection in Ghana (52%) (Hotez *et al.*, 2003). Infection with *Ascaris* has been reported to vary between relative rates of 1.5-18.0 in children and relative risks of 3.5-5.4 in adults. Even in cases of helminth egg loads of <1 in 1L of water children were still found to be at a higher risk (Cifuentes *et al*., 2000). Farmers and their households (especially children) engaged in wastewater irrigation are at a higher risk of helminth infection due to the duration and intensity

of their contact with the wastewater and contaminated soils. Consumers of vegetables irrigated with wastewater, especially vegetables that are eaten without cooking (cabbage and lettuce) before consumption are also at risk of infection with helminths found in the wastewater especially *Ascaris*. Therefore there is the need to determine the risk of infection with *Ascaris* for the farmers and consumers. Considering the three different methods generally used to evaluate microbial risks, only microbiology laboratory analysis and epidemiological studies have been applied to helminth data.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wastewater use in Irrigated Agriculture

Fresh water is a scarce commodity in many parts of the world, with population growth in semi-arid and arid regions would further increase the scarcity of this resource. Growing competition for freshwater especially in water scarce regions would greatly increase the pressure on this important resource (WHO, 2006). For example the UN predicts that most of the 19 cities expected to grow rapidly during 2000-2015 are in chronically water scarce regions of developing countries (United Nations Populations Division, 2002). Wastewater is approximately 99% of water (WHO, 2006), The water and nutrient value serves as important resources for farmers in both developed and developing countries. A large proportion of vegetables sold within most cities especially in developing countries is produced using wastewater in urban and peri-urban area (Amoah, 2009). For example, Faruqui *et al* (2004) reported that in Dakar, Senegal, more than 60% of the vegetables consumed in the city are grown within urban areas with the use of a mixture of groundwater and untreated wastewater. In Kumasi, wastewater is mostly use in a diluted form, often mixed with surface runoff and stream water (Keraita *et al*., 2003)

2.2 Opportunities associated with wastewater irrigation

The water and nutrient value of wastewater make it an important resource for farmers, wastewater flow is often reliable and available all year round. (Gleick, 2003). Parameswaran (1999) has demonstrated the positive impact of wastewater on crop production as a result of its nutrient content and organic matter. The use of wastewater leads to a reduction in the use of artificial fertilizers, therefore forming an important part in nutrient recycling. Thus supplementary fertilization needs can be reduced for some crops or even eliminated for others

with a subsequent increase in the income of the farmer. Irrigation with wastewater produces higher crop yield than irrigation with fresh water, even with the use of artificial fertilizers. For example in India, irrigation with waste stabilization pond effluents yielded 28,8,47,30 and 42 % more wheat, moong beans, rice, potato and cotton respectively, than irrigation with fresh water supplemented with fertilizer (Shende *et al.*, 1985). Similar results were also obtained from irrigation of vegetables with wastewater instead of irrigation with pipe water. This practice also shortens the production time of the vegetables (e.g. lettuce) (Faruqui *et al.*, 2004). Higher crop yields means improve food availability. With increase in the supply of food market prices are lowered. Therefore more people can afford to buy food thereby improving their nutrition status. This therefore has the potential of helping us to reach the Millennium Development Goal 1 (MDG 1), which states "eliminate extreme poverty and hunger".

2.3Microbial hazards in Wastewater

2.3.1 Irrigation water

Despite the potential benefits, it is very important to be aware of the health hazards that may result from the re-use of wastewater in irrigated farming. Wastewater is a potential carrier of bacteria, viruses, protozoa and nematodes, which can cause various diseases (Asano and Cortuvo, 2004). The problem of microbial pollution becomes more serious with the vegetables, because many of them are being consumed raw. However, the extent of the pollution decreases if the vegetable's edible plant parts are above the ground, while it increases if they are near the ground (Minhas and Samra, 2004; Al-Lahham *et al.*, 2003). In addition to the health

threat posed to consumers of the produce from these farms, the farmers, their families and other farm workers are at a greater health risk of infection with pathogens due to their constant contact with the wastewater. 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 period 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 cultivated 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. Some are aware of such measures but cannot afford protective gadgets or give them little priority. No extension services are offered to farmers on irrigation practices and related protection, etc. 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 (Bethony *et al.*, 2006). 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).

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 (Cifuentes *et al*, 1993).

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.

Toze (1997) divided microbial pathogens which can be potentially present in soil and wastewater into three separate groups. These groups are the viruses, bacteria and the pathogenic protozoan/helminthes. Gerardi and Zimmerman (2005) included fungi as a fourth group. But enteric pathogens transmitted by the fecal–oral route are usually bacteria, viruses, protozoa and helminthes (Santamaria and Toranzos, 2003). These pathogens are the causative agents of bacterial, viral and protozoan diseases endemic in the community and excreted by diseased and infected individuals (Shuval *et al.*, 1986).

Most pathogenic microbial agents found in wastewater are enteric in origin i.e. they are excreted in faecal matter, contaminate the environment and enter new hosts through ingestion (Toze, 1997). These microbes get into the environment through the faeces of infected hosts and can enter surface water through run-off from soil and other land surfaces, direct defecation into water, and contamination with sewage effluent (Feachem *et al.*, 1983). The numbers and types of pathogens found in wastewater vary both spatially and temporally depending on season, water use, economic status of the population, disease incidence in the population producing the wastewater, awareness of personal hygiene, and quality of water or food consumed (WHO, 2006). Table 1 gives a summary of the major pathogens in wastewater and their concentrations.

Table 1: Pathogen concentration in wastewater

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

ND, no data

SOURCE:WHO (2006)

2.4: Wastewater irrigated Soil

In the preparation of soil for planting, farmers mostly use un-composted organic manures to fertilize the soil. These manures contain several microorganisms (Amoah, 2009). Soil is known to contain a variety of microorganisms. 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. Of all the organic manures however, poultry manure seems to be the cheapest and the most preferred. 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. These pathogenic organisms can therefore pose a health threat to the farmers. Of particular health importance is the transmission of intestinal helminths often referred to as Soil-transmitted Helminths (STHs). Soil-transmitted helminth infections are among the most common infections worldwide and affect the poorest and most deprived communities. They are caused by parasitic worms (helminths) that are transmitted to people through contaminated soil. The main species of soil-transmitted helminths that infect people are the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and the hookworms (*Necator americanus* and *Ancylostoma duodenale*). Soil-transmitted helminths are transmitted by eggs that are passed in the faeces of infected people. Adult worms live in the intestine where they produce thousands of eggs each day. In areas that lack adequate sanitation, these eggs contaminate the soil. People become infected with *A. lumbricoides* and *T. trichiura* by ingesting infective parasite eggs. This can happen in several ways.

- Eggs that are attached to vegetables are ingested when the vegetables are not carefully cooked, washed or peeled.
- Eggs are ingested from contaminated water sources.
- Eggs are ingested by children who play in soil and then put their hands in their mouths without washing them.

2.5 PARASITES IN WASTEWATER AND WASTEWATER IRRIGATED VEGETABLES

Water plays a major role in mobilizing and transporting microorganisms. Rainfall washes organisms from faeces or vegetation surfaces and directs run-off water into soils or along the land surface into surface water. Several 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 (Cai and Zhang, 2013).

2.5.1Helminths:

Soil transmitted helminthes are commonly known as intestinal worms. They are the most common infections worldwide affecting the most deprived communities. Helminths include nematodes and tape worms. They are common intestinal parasites which are transmitted through the faecal-oral route (Santamaria and Toranzos, 2003). Intestinal nematodes are the greatest health risk involved in the use of untreated wastewater in agriculture (WHO, 1989). For instance, helminth infections cause heavy blood losses, and anaemia and retardation in children (Ensink*et al.,* 2004). Some of the helminth parasites require an intermediate host for development prior to

becoming infectious for humans (Toze, 1997). Some of the commonly detected parasites that are of significant health risk, include round worm (*Ascaris lumbricoides*), the hook worm (*Ancylostoma duodenale or Necator americanus)*, the causative agent of strongyloidiasis (*Strongyloides stercoralis*), and the whip worm (*Trichuris trichiura*).

Helminth infection levels are particularly endemic where human faecal matter is used as a fertilizer for growing vegetables (Khuroo, 1996). Approximately 25% of the world's human population is infected with *Ascaris lumbricoides* (Ellis *et al.*, 1993). *Ascaris lumbricoides*is endemic in regions of Asia, India, South America and Africa (Khuroo, 1996). The type of helminth infection is dependent on environmental and socio-economic conditions (Toze, 1997). One instance is the case of *Strongiloides stercoralis,* a soil transmitted parasitic nematode endemic in northern Australia (Fisher *et al.,* 1993). Helminth eggs require moist shady soil for embryonation of the eggs over a period of five to ten days before they are able to cause infection (Toze, 1997). Following embryonation, however, the eggs can remain infectious in the contaminated soil for up to ten years (Khuroo, 1996). This means that any soils which have been in contact with recycled waters contaminated with faecal material could be considered as potential long-term sources of these parasites (Ellis *et al.*, 1993 WHO, 1989). Soil-transmitted helminthes produce a wide range of symptoms including intestinal manifestations (diarrhea, abdominal pain), general malaise and weakness, which may affect working and learning capacities and impair physical growth. Hookworms cause chronic intestinal blood loss that result in anaemia (WHO, 2006).

2.5.1.1. 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. Hookworm infection of human beings occurs through the penetration of the filariform larvae through the feet (Figure 1). The eggs are passed through the feaces of infected people and then hatch in the soil into rhabditiform larvae which then develops into the filariform larvae (Figure 1).

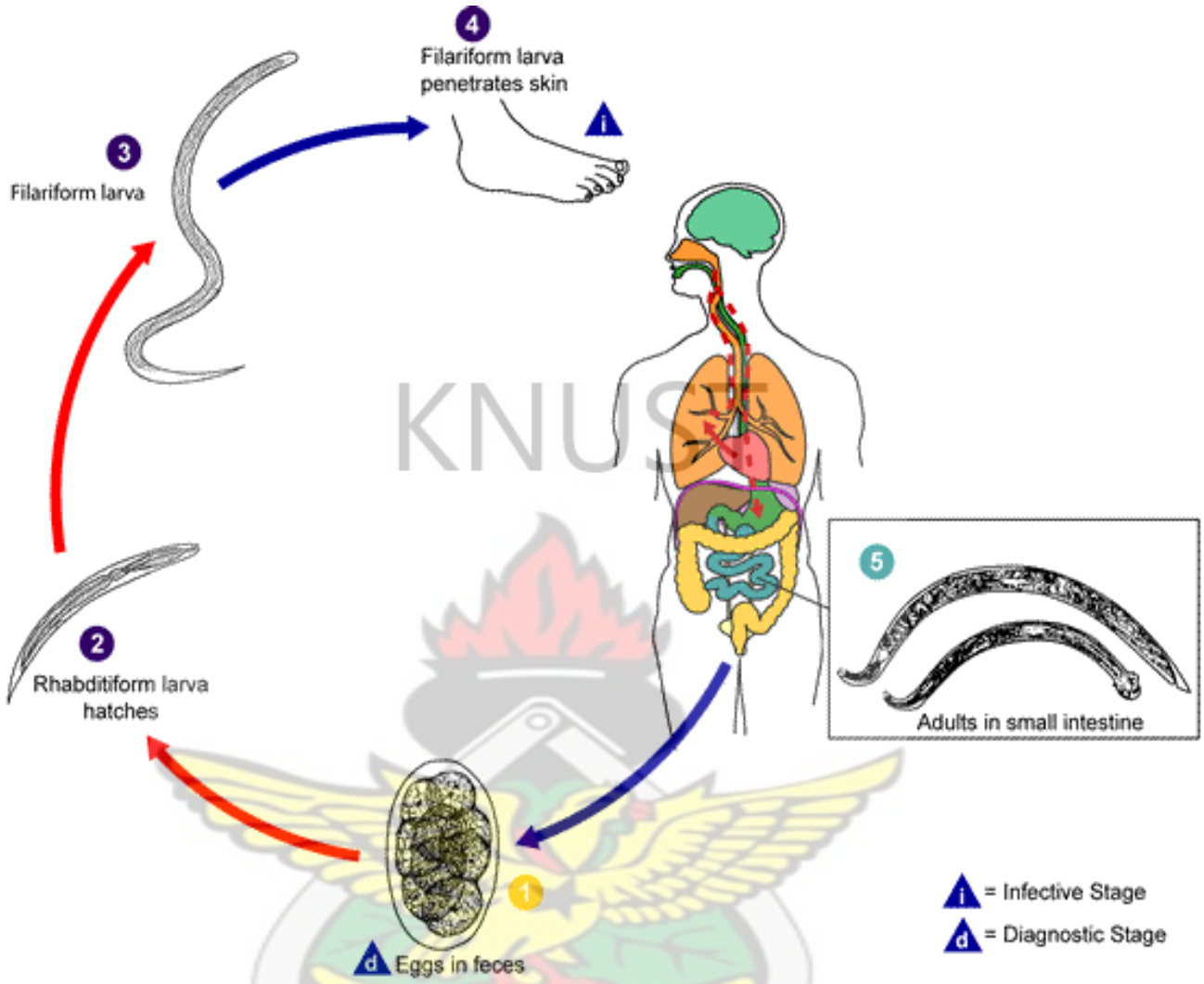

Fig. 1 Life Cycle and Transmission of Hookworm

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

2.5.1.2 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 swallowed 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.

About 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 was used to fertilize crop fields, 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. Adult worms (Fig. 2) live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the feces (Fig. 2). Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks (Fig. 2), depending on the environmental conditions (optimum: moist, warm, shaded soil). After infective eggs are swallowed , the larvae hatch, invade the intestinal mucosa, and are carried via the portal, then

systemic circulation to the lungs. 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 .

Upon reaching the small intestine, they develop into adult worms (Fig. 2). 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.

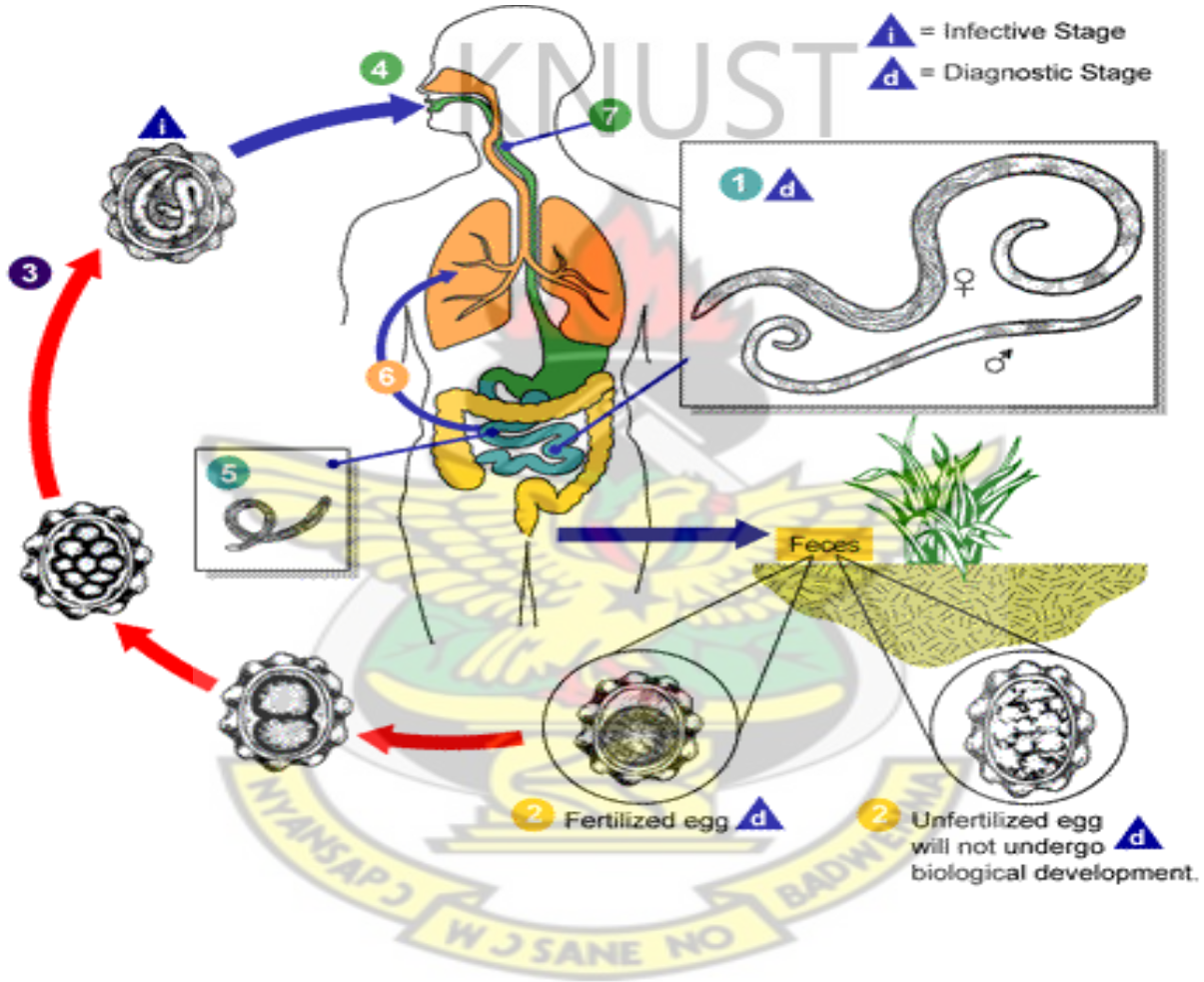

Fig. 2: Life Cycle and Transmission of *Ascarislumbricoides*

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

2.5.1.3 Trichuristrichuira

It is the third most common round worm of humans, with infections more frequent in areas with tropical weather and poor sanitation practices, especially in Asia and, to a lesser degree, in Africa and South America., and among children. It is estimated that 800 million people are infected worldwide. There is a worldwide distribution of *Trichuris trichiura*, with an estimated 1 billion human infections. Poor hygiene is associated with trichuriasis as well as the consumption of shaded moist soil, or food that may have been fecally contaminated. Children are especially vulnerable to infection due to their high exposure risk. Eggs are infective about 2–3 weeks after they are deposited in the soil under proper conditions of warmth and moisture, hence its tropical distribution. The eggs hatch in the small intestine and then move into the wall of the small intestine and develop. On reaching adulthood, the thinner end (the front of the worm) burrows into the large intestine and the thicker end hangs into the lumen and mates with nearby worms. The females can grow up to 50 mm (2 inches) long. Neither the male nor the female has much of a visible tail past the anus. Whipworm commonly infects patients also infected with *Giardia*, *Entamoeba histolytica*, *Ascaris lumbricoides*, and hookworms. Infection with this parasite is through the ingestion of the embryonated eggs which then hatch into larvae in the small intestine, mature into adults in the cecum. The adult worms then lay eggs that are passed out in feaces (Fig. 3)

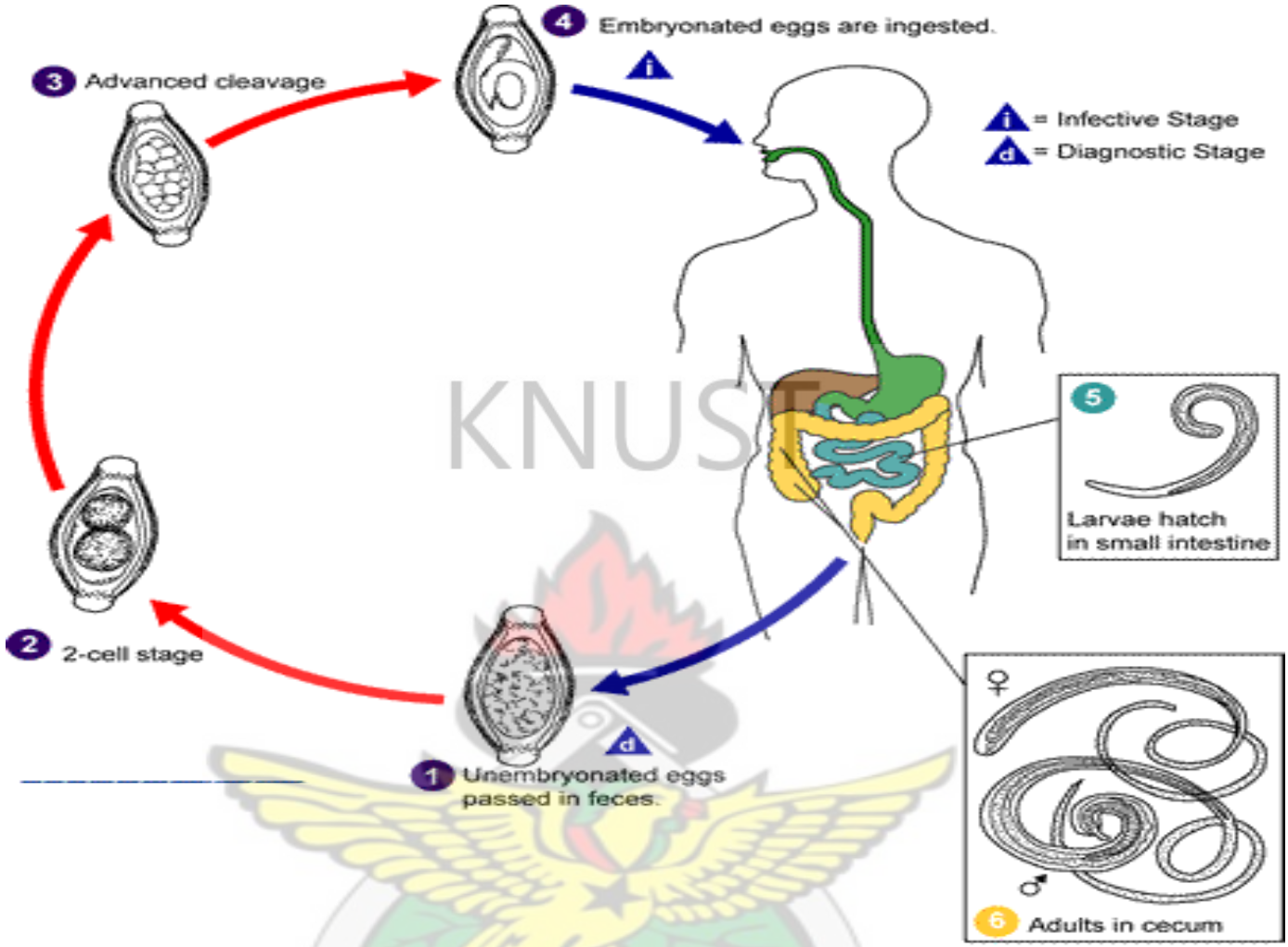

Fig. 3 Life Cycle and Transmission of *Tichuristrichiura*

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

2.5.1.4 Schistosomaspp

Schistosomes have a typical trematode vertebrate-invertebrate lifecycle, with humans being the definitive host. The life cycles of all five human schistosomes are broadly similar: parasite eggs are released into the environment from infected individuals, hatching on contact with fresh water to release the free-swimming miracidium. Miracidia infect freshwater snails by penetrating the

snail's foot. After infection, close to the site of penetration, the miracidium transforms into a primary (mother) sporocyst. Germ cells within the primary sporocyst will then begin dividing to produce secondary (daughter) sporocysts, which migrate to the snail's hepatopancreas. Once at the hepatopancreas, germ cells within the secondary sporocyst begin to divide again, this time producing thousands of new parasites, known as cercariae, which are the larvae capable of infecting mammals. Cercariae emerge daily from the snail host in a circadian rhythm, dependent on ambient temperature and light. Young cercariae are highly mobile, alternating between vigorous upward movement and sinking to maintain their position in the water. Cercarial activity is particularly stimulated by water turbulence, by shadows and by chemicals found on human skin. Penetration of the human skin occurs after the cercaria has attached itself to the skin. The parasite secretes enzymes that break down the skin's protein to enable penetration of the cercarial head through the skin. As the cercaria penetrates the skin it develops into a migrating schistosomulum stage. The newly transformed schistosomulum may remain in the skin for two days before locating a post-capillary venule; from here the schistosomulum travels to the lungs where it undergoes further developmental changes necessary for subsequent migration to the liver. Eight to ten days after penetration of the skin, the parasite migrates to the liver sinusoids. *S. japonicum* migrates more quickly than *S. mansoni*, and usually reaches the liver within eight days of penetration. Juvenile *S. mansoni* and *S. japonicum* worms develop an oral sucker after arriving at the liver, and it is during this period that the parasite begins to feed on red blood cells (Fig. 4). The nearly-mature worms pair, with the longer female worm residing in the gynaecophoric channel of the shorter male. Adult worms are about 10 mm long. Worm pairs of *S. mansoni* and *S. japonicum* relocate to the mesenteric or rectal veins. *S. haematobium*

schistosomula ultimately migrate from the liver to the perivesical venous plexus of the bladder, ureters, and kidneys through the hemorrhoidal plexus.

Parasites reach maturity in six to eight weeks, at which time they begin to produce eggs. Adult *S. mansoni* pairs residing in the mesenteric vessels may produce up to 300 eggs per day during their reproductive lives. *S. japonicum* may produce up to 3,000 eggs per day. Many of the eggs pass through the walls of the blood vessels, and through the intestinal wall, to be passed out of the body in feces. *S. haematobium* eggs pass through the ureteral or bladder wall and into the urine. Only mature eggs are capable of crossing into the digestive tract, possibly through the release of proteolytic enzymes, but also as a function of host immune response, which fosters local tissue ulceration. Up to half the eggs released by the worm pairs become trapped in the mesenteric veins, or will be washed back into the liver, where they will become lodged. Worm pairs can live in the body for an average of four and a half years, but may persist up to twenty years.

Trapped eggs mature normally, secreting antigens that elicit a vigorous immune response. The eggs themselves do not damage the body. Rather it is the cellular infiltration resultant from the immune response that causes the pathology classically associated with schistosomiasis.

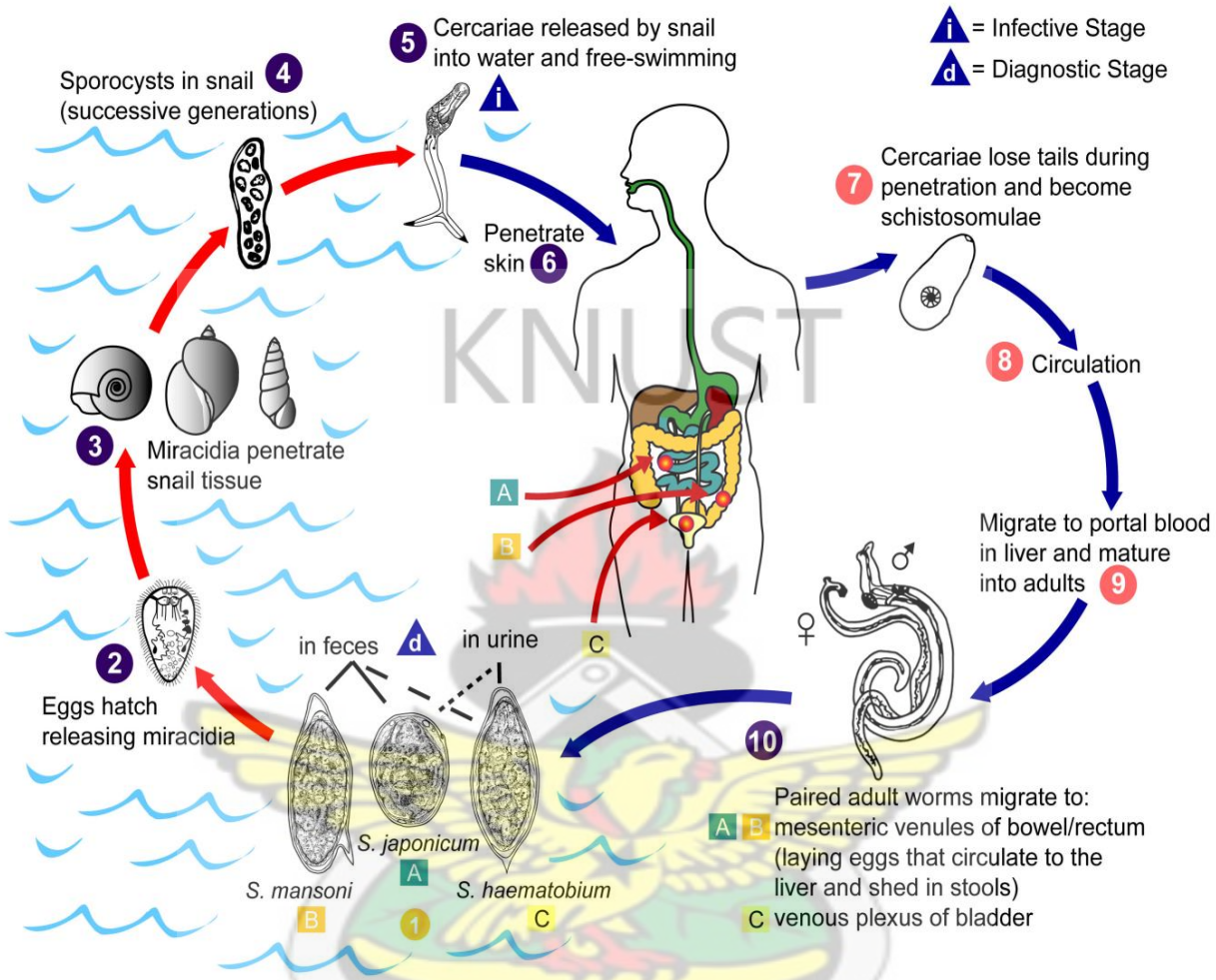

Fig. 4 Life Cycle and Transmission of *Schistosomaspp*

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

2.6 Survival and persistence of pathogens in soil, crops and wastewater

The ability of an excreted organism to survive outside the human body is referred to as its persistence (Wescott, 1997). Toze (1997) stated that the persistence or survival of pathogenic

microorganisms, and their resistance to treatment processes is an important wastewater issue. This survival of the pathogens can be related to the potential microbial species involved in the wastewater applications and health risk analysis. Pathogenic microorganisms remain a health risk as long as they persist in environments such as in wastewater. The longer they survive in an environment the greater the potential they have of becoming infective if the chemical, physical or prevailing water conditions are suitable. Increased persistence and survival also increases the chance of their spreading, for example through spray irrigation. Therefore, the longer pathogens persist in wastewater, the greater the chance that they could come into contact with workers and the general public. The survival of pathogens is affected by several factors, including; the type of organism, the presence of other antagonistic organisms, the soil characteristics, temperature, moisture, nutrients, pH, and sunlight. Wide variability in survival times reflects the influence of environmental factors (Wescott, 1997). Some organisms are more resistant than others. Soil moisture favours the survival of viruses and bacteria (Santamaria and Toranzos, 2003). Soil moisture content of about 10 to 20 percent appears to be best for survival (Wescott, 1997). Reduction in bacterial and viral population, are observed under dry soil conditions. Exposure to sunlight increases the death rate as the ultraviolet light from the sun inactivates pathogens on the surface of the soil but pathogens in deeper layers are not affected (Toze, 1997). Viral survival may be longer than bacterial and longevity is greatly increased at lower temperatures (Wescott, 1997). Generally, lower temperatures favor pathogen survival (Toze, 1997; Santamaria and Toranzos, 2003). The adsorption of pathogen cells to clay has been demonstrated to be advantageous to their survival. Clays favor the adsorption of microorganisms to soil particles and this further reduces the die-off rates (Gerba and Bitton, 1984; Yeager and Ward, 1981). Clays protect bacteria cells, and possibly viral particles, by creating a barrier against microbial

predators and parasites (Santamaria and Toranzos, 2003). Hence, survival rates of enteric pathogen are lower in sandy soils with a low water-holding capacity. Santamaria and Toranzos (2003) stated that pH affects the adsorption characteristics of cells, so inactivation rates in acidic soils are lower. Increases in cation concentrations also results in increased adsorption rates, consequently affecting microbial survival (Santamaria and Toranzos, 2003). They also mentioned that soluble organics increase survival and, in the case of bacteria, may favor their re-growth when degradable organic matter is present. Helminth eggs, in some cases, can survive for several years in the soil and wastewaters (Parsons *et al.*, 1975; Toze, 1997) and can remain viable on crop surfaces for up to two months, although a few survive beyond approximately 30 – 35 days (Strauss, 1996). Knowledge of the survival of pathogens in soil and on the crop allows an initial assessment of the risk of transmitting disease via produced foodstuff or through worker exposure (Wescott, 1997). The survival times of the pathogens in water are different from that of the soil and crops. Almost all excreted pathogens can survive in soil for a sufficient length of time to pose potential risks to farm workers (WHO, 1989). Pathogens survive on crop surfaces for a shorter time than in the soil as they are less well protected from the harsh effects of sunlight and desiccation. Nevertheless, survival times can be long enough in some cases to pose potential risks to crop handlers and consumers, especially when survival times are longer than the crop growing cycles as is often the case with vegetables. The exposed pathogens, if they do enter an irrigated area with the irrigation water, have the potential to remain infectious for a considerable period of time therefore steps must be taken to interrupt this infection cycle WHO (1989).

2.7. SOURCES OF CONTAMINATION OF VEGETABLES

Parasitic pathogens can contaminate vegetables through various routes, either during pre-harvest, harvest and post-harvest. For example, through feacally contaminated water used for irrigation and poor hygienic practices during crop handling further feacal matter can contaminate the vegetables. These examples make it possible for parasitic pathogens from human and animal reservoirs as well as from the environment contaminate crops at the point of consumption (Table 2 shows the source of contamination for vegetable from farms).

TABLE 2: Sources of contamination of vegetables.

Pre-Harvest	Post-harvest	Sorting, packaging and further processing equipment.
• Feaces • Soil • Irrigation water • Green or inadequately composted manure • Air (dust) • Wild and domestic animals • Human handling	• Feaces • Human handling • Harvesting equipment • Transport containers • Wild and domestic animals • Insects • Air(dust) • Wash and rinse water	• Ice • Transport • Improper storage • Improper packaging • Cross contamination • Improper display temperature • Improper handling after wholesale or retail purchase.

Data after Beauchat and Ryu (1997).

2.7.1. Pre-harvest

The main route through which vegetables can be contaminated with parasitic pathogens during the pre-harvest stage is either through the soil, wastewater or the organic fertilizers used. The soil is a reservoir of various parasitic pathogens, these are mostly found in the soil due to irrigation and fertilization with manure and sludge or due to droppings of animals in the farming area. This contamination could also be through the wastewater used for the irrigation, which has been shown to contain various parasitic protozoans. Surface water from streams and lakes may be contaminated with pathogenic protozoans. In attempts to improve yield farmers resort to fertilizers, but due to the high cost of inorganic fertilizers, most of the farmers resort to organic fertilizers. These organic fertilizers are from animal sources and therefore contain a lot of parasitic pathogens which could end up on the vegetables due to splashing during irrigation and through other means.

2.7.2. 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, 1999), 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.

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.

2.7.3. 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.

2.9. 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).

Amoah *et al* ., (2005) reported that food vendors employ various decontamination methods in an attempt to reduce the level of pathogens on lettuce. Some of these methods includes washing in; tap water in a bowl (no sanitizer), running tap water, salt solution, vinegar solution and potassium permanganate solution. This study reported that all treatments employed could at least reduce helminth egg population by half.

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) (Amoah *et al*., 2005).

2.10. Microbial hazards transmission pathways

The use of wastewater for irrigation poses various health risks, due to the presence of pathogens in the wastewater, key among these are *Salmonella*, *E. coli* and intestinal nematodes *like A. lumbricoides, T. trichuira , Ancylostoma duodenale* and *Necator americanus*. The most prevalent parasitic infection worldwide has been reported to be *A. lumbricoides* with infection rates ranging from 40-98% in Africa. Association between ascariasis and wastewater use among farmers have been established by several studies (Seidu *et al.*, 2008). Ascariasis prevalence in Ghana has been found to be 52%. Pathogens associated with wastewater irrigation are ingested

orally or through penetration of the skin. Farm workers are at a higher risk of infection with *A. lumbricoides* and hookworm due to the duration and intensity of their contact with wastewater and contaminated soils, however children engages in wastewater irrigation are disproportionately affected (Seidu *et al.*, 2008).

The use of wastewater for irrigation also has serious health implications for consumers of the produce, and people living in close proximity to these irrigation sites. Most vegetables sold in urban areas are produced with wastewater and therefore exposing the consumers to varying levels of infection risk based on the quality of the wastewater used for the irrigation as wells as, treatments during and after harvest, transportation, storage and the food preparation stage. Various interventions have been documented to cause a 1-3 log reduction in *A. lumbricoides* concentrations (Amoah *et al.*, 2007).

2.11. Health Risks

While recycling and re-use of wastewater for agriculture, industry and non-potable urban purposes can be a highly effective strategy for developing a sustainable water resource in water deficient areas, nutrient conservation and environmental protection, it is essential to understand the health risks involved and to develop appropriate strategies for the control of those risks (Shuval *et al.*, 1986). The detection of pathogens in soil, wastewater used for irrigation and on crops indicates potential environmental and health risks to occupationally exposed farmers and consumers of the contaminated crops. There are soil-borne diseases caused by enteric pathogens which get into soil by means of human or animal excreta (Weissman *et al.*, 1976).

The re-use of wastewater for irrigated agriculture worldwide has been approached with a degree of trepidation, owing to primary concerns about the risks to human health via contamination of

food through pathogenic microorganisms (Hamilton *et al.*, 2005). The major threat to farmers and their families is from intestinal parasites – most often worms (Faruqui *et al.*, 2004. Living in the small intestine, hookworms cause heavy blood losses, and anaemia and retardation in children (Ensink *et al.*, 2004). Bacterial and viral infections are other health threats which can occur after the consumption of raw vegetables contaminated with faecal matter. Lastly, health risks vary according to gender, class, and ethnicity (Buechler, 2004). In both Latin America and South Asia, women often perform the tasks requiring the most extensive contact with wastewater, such as transplanting and weeding in flooded areas like paddy fields (Faruqui *et al.*, 2004). Furthermore, the children of farmers or farm workers, who have not yet built up immunity, tend to be most at risk to gastrointestinal problems (Faruqui *et al.*, 2004).

In other studies of the risks of *Ascaris* infection to farm workers and their families related to the use of treated wastewater it was suggested that there was an increased risk of *Ascaris* infection in children even when the quality of the wastewater was ≤1 nematode egg per litre. With a further suggestion that the threshold may be above 1 nematode egg per litre for adults. The low infectious dose for *Ascaris*, and the persistence of eggs in the environment could explain the low threshold level (Ensink *et al.*, 2004). The health risk due to exposure to wastewater irrigation differ depending on the exposed group. Table 3 shows the various health risks and the particular group at risk.

Table 3: Health risks due to wastewater irrigation for different exposed groups.

Group exposed	Health risks		
	Helminth infections	**Bacterial/virus infections**	**Protozoal infections**
Consumers	Significant risk of *Ascaris* infection for both adults and children with untreated wastewater	Cholera, typhoid and shigellosis outbreaks reported from use of untreated wastewater; seropositive responses for *Helicobacter pylori* (untreated); increase in non-specific diarrhoea when water quality exceeds 10^4 thermotolerant coliforms/100 ml	Evidence of parasitic protozoa found on wastewater-irrigated vegetable surfaces, but no direct evidence of disease transmission
Farm workers and their families	Significant risk of *Ascaris* infection for both adults and children in contact with untreated wastewater; risk remains, especially for children, when wastewater treated to <1 nematode egg per litre; increased risk of hookworm infection in workers	Increased risk of diarrhoeal disease in young children with wastewater contact if water quality exceeds 10^4 thermotolerant coliforms/100 ml; elevated risk of *Salmonella* infection in children exposed to untreated wastewater; elevated seroresponse to norovirus in adults exposed to partially treated wastewater	Risk of *Giardia intestinalis* infection was found insignificant for contact with both untreated and treated wastewater; increased risk of amoebiasis observed with contact with untreated wastewater
Nearby communities	*Ascaris* transmission not studied for sprinkler irrigation, but same as above for flood or furrow irrigation with heavy contact	Sprinkler irrigation with poor water quality (10^6–10^8 TC/100 ml) and high aerosol exposure associated with increased rates of infection; use of partially treated water (10^4–10^5 thermotolerant coliforms/100 ml or less) in sprinkler irrigation not found to be associated with increased viral infection rates	No data on transmission of protozoan infections during sprinkler irrigation with wastewater

TC, total coliforms

Source: WHO (2006)

2.11.1 Exposure of farmers to helminth infection

According to the WHO (2006) there is evidence to suggest that direct contact with untreated wastewater can result in increased helminth infection (mainly *Ascaris* and hookworm). This risk of infection also varies depending on the type of irrigation practice. When flood or furrow irrigation is used, the effect of direct contact with untreated wastewater on *Ascaris* infection varies according to area and initial prevalence (Bouhoum and Schwatzbrod, 1998; Habbari *et al*., 2000; Blumenthal *et al*., 2001). The effect of exposure to untreated wastewater on hookworm infection varies from attributable risks of between 37% (Krishnamoorthi, Abdulappa and Anwikar, 1973) and 14% (Ensink *et al*., 2005) in adults.

Infection with *Ascaris* can be reduced when wastewater is partially treated before use. Peasey (2000) found from studies in Mexico that where wastewater retention was ensured in a single reservoir for a minimum of one month during the year preceeding the study, there was a 2 log nematode removal; there was no increased risk of *Ascaris* infection for adults, but still significant in children (Peasey, 2000;Blumenthal *et al.*, 2001).

2.11.2. Exposure of consumers to helminth infection

The greatest health risks for consumers of wastewater irrigated produce are with crops eaten raw, for example salad crops, especially root crops or crops close to the soil surface (lettuce) (WHO, 2006). Susceptibility of crops to contamination varies. For example onions (Blumenthal *et al*., 2003) and lettuce (Solomon *et al.*, 2002) are more susceptible. Stine *et al.*, (2005) reported that lettuce and cantaloupe surfaces retain pathogens from irrigation water spiked with *E. coli* and bacteriophage (PRDI).

An estimated 70% of street food consumers prefer a salad component. In cafeterias an estimated 60% prefer salads. Previously, salad was mainly sold as a component of exotic meals like rice. In recent times most Ghanaians take salad along with local dishes like "awaakye", " gari and beans" etc (Fung, 2011). This has lead to an increase in salad consumption. At least three kinds vegetables are mixed to make a salad, with lettuce and cabbage being the main components, Carrots, onions, tomatoes, cucumber and green pepper are in some cases added but in relatively smaller quantities. These vegetables used for the preparation of salad come from farms located in and around major cities where wastewater is the source of irrigation water. Therefore the consumers were exposed to helminth infection. Consumers are exposed to varying levels of infection depending on the exposure pathway, namely the type of irrigation water used, farming practices employed, harvesting techniques, storage, transportation, marketing conditions and the level of hygiene and interventions during the preparatory stage. These conditions and the amount of vegetables consumed on a daily basis determines the risks of helminth infection for consumers of wastewater irrigated vegetables.

Studies by Peasey (2000) produced adjustable odds ratios (OR) of 3.9 (men) and 2.4 (children) due to the consumption of vegetables irrigated with wastewater by farming families. When this study took into account other contributing factors for *Ascaris* infection such as the age, gender, socioeconomic status and direct wastewater contact, the attributable risk from consumption was 25% for children and 14% for adult men.

2.11.3 Health risk of people living in close proximity to wastewater irrigation sites

Human beings come into contact with the wastewater (or contaminated crops) before, during or after irrigation, as well as inhalation of wastewater aerosols. Shuval *et al.* (1989) found that

occurrence of enteric disease were similar in communities most exposed to treated wastewater aerosols from stabilization ponds and in those not exposed to wastewater in any form. Serological studies in Isreal (Fattal *et al*., 1985, 1987; Margalith *et al.*, 1990) suggests that exposure to aerosols through sprinkler irrigation from 5 to 10 day waste stabilization ponds does not relate to excess endemic viral infection. The risks of helminth infection for communities close to the irrigation sites is reported where there is direct contact with the wastewater where furrow irrigation is practiced (WHO, 2006).

Vector borne disease transmission resulting from the development and management of wastewater irrigation schemes and waste stabilization ponds is one major health concern for communities close to wastewater irrigation sites. Dengue fever, filariasis, Japanese encephalitis and malaria are some of the diseases transmitted through wastewater irrigation, as shown in Table 4. Studies conducted in Pakistan by Mukhtar *et al*., (2006) found evidence to suggest that wastewater irrigation plays a significant role in the breeding of mosquitoes which serve as vectors for various diseases. In Ghana, investigation of the impact of urban agriculture on malaria vectors in Accra, showed that urban malaria is increased in areas where irrigated farming takes place (Klinkerberg *et al.*, 2008).

Table 4: Summary of the vector borne diseases associated with wastewater irrigation

Disease	Vector	Relative risk of wastewater use in agriculture	Comments
Dengue	*Aedes aegypti*	Low	Vectors breed in standing water (e.g. tires, cans, bottles, etc.). Present in South-east Asia but not China.
Filariasis	*Culex quinquefasciatus*	Medium	Vectors breed in organically polluted water. Endemic in many countries where wastewater use in agriculture is practised.
Japanese encephalitis	*Culex* spp.	Medium	Vectors breed in flooded rice fields. Endemic in many countries where wastewater use in agriculture is practised.
Malaria	*Anopheles* spp.	Low	Vectors breed in uncontaminated water; 90% of malaria cases occur in Africa. *Anopheles* breeding has been reported from serial waste stabilization ponds.

Source: WHO (2006)

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

This study was conducted in wastewater irrigated vegetable farms in the Kumasi Metropolis (Fig. 1) in the Ashanti Region of Ghana. Kumasi is the second largest and one of the fastest growing cities in Ghana with an estimated population of a little over 2 million and an annual growth rate of 2.7% (Ghana Statistical Service, 2010). It lies between Latitude 6° 41'13.63'' North and Longitude 1°37'19.09'' West with average altitude of 287m. It covers a total area of 254 km^2. The metropolis 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 from 60% to 84.16% with daily minimum and maximum temperatures of 21.50°C and 30.70°C, respectively (MOFA, 2013).

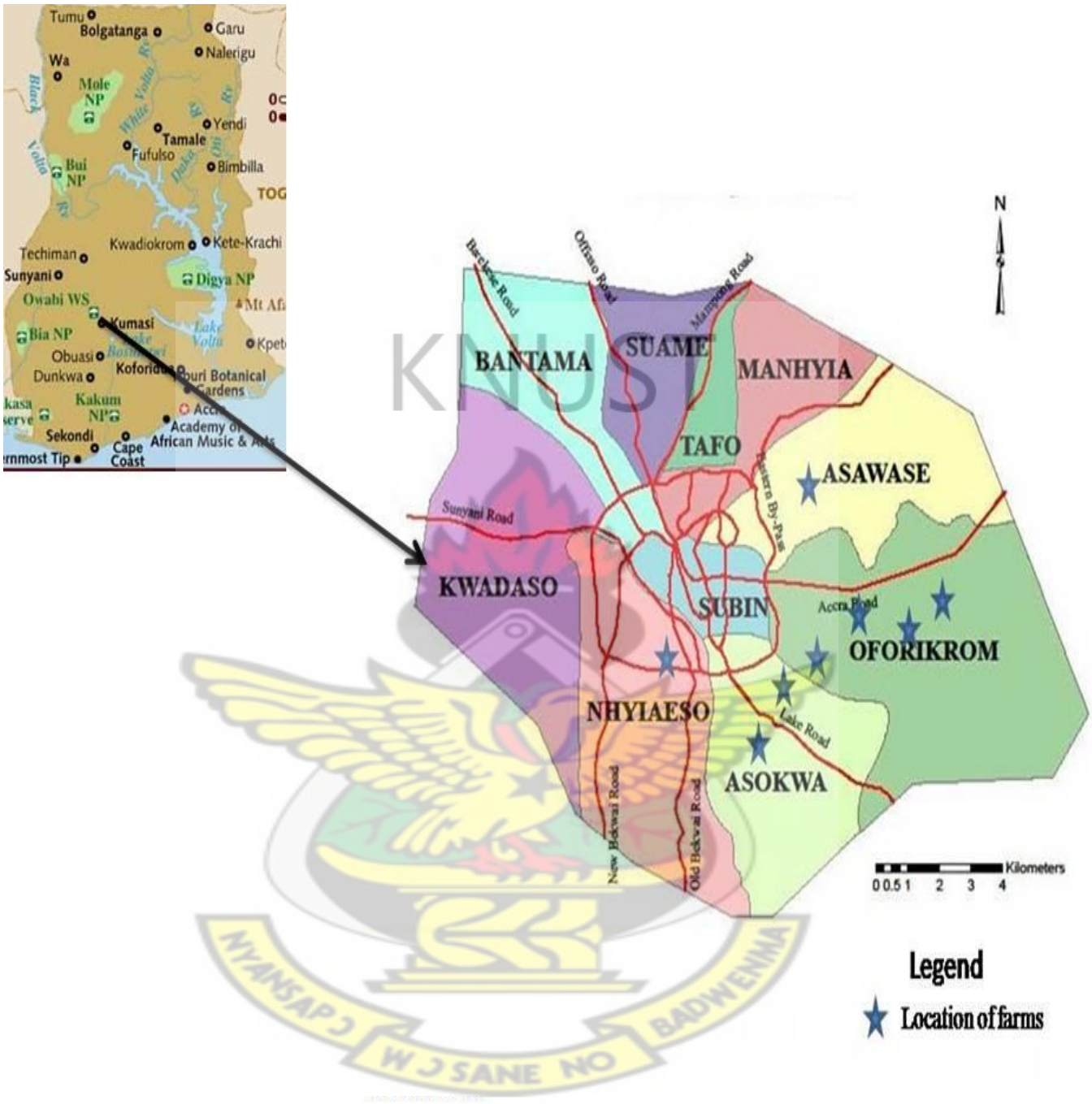

Figure 1: Map of Ghana showing the Kumasi Metropolitan Area and the wastewater irrigated farms.

3.2 SAMPLING SITES

All vegetable farms within the Kumasi Metropolis were surveyed and grouped into eight (8) sampling sites based on location and the use of wastewater for irrigation, namely:

- PokuSika (consists of farms within KNUST Campus),
- Ayeduase (farms within, Ayeduase, Kotei, Deduako and Boadi),
- Gyinyase,
- Ramseyer (farms located within the Presbyterian Vocational Institute, Chirapatre),
- Apemso/Appiadu (these are farms located within the Apemso and Appiadu communities),
- Kentinkrono,
- Georgia (farms located close to Georgia Hotel, Ahodwo) and
- Nima (farms located within Aboabo).

Irrigation water and soil samples from these farm sites were randomly collected. The irrigation water was sampled from water sources used by the farmers to irrigate the vegetable beds. The water sources from which samples were taken included streams, shallow wells, storm drains and pipe-borne water (used in few instances and included in the study as a control). Soil samples were collected from vegetable beds.

3.3 SAMPLING

3.3.1 Irrigation water and soil

In the wet season samples were collected from August 2012 to November 2012. Dry season samples were collected from December 2012 to March 2013. All samples were collected in the

morning between the hours of 0600GMT to 1000GMT on each day of sampling. Irrigation water samples were collected in triplicates into sterile pre-labeled sample bottles (about 4L) from each selected irrigational water source. Soil samples were taken in triplicates, about 30g each, from vegetable beds to represent the different irrigational water sources. All samples were kept in a cooling box and transported to the laboratory where they were processed and analyzed for helminth eggs using the Modified EPA Method (Schwartzbrod, 1998).

3.3.2 Salad foods

An initial survey was conducted to identify salad food sellers in all the ten sub-metros of the Kumasi Metropolitan Area. Salad food sellers were then randomly selected. Sampling was done between the hours of 16:00GMT and 18:00GMT daily. Collected samples were kept in a cooling box and transported to the laboratory for analysis.

3.4 HELMINTH IDENTIFICATION

Prior to the use of the Modified EPA method (Schwartzbrod, 1998) which combines both the flotation and sedimentation techniques, the various samples were processed as follows;

3.4.1. Irrigation water: The 2L of each sampled water was poured in a container of about 4L and then taken through the modified EPA protocol as elaborated below (Section 3.5).

3.4.2. Soil: The sampled soil was weighed (30g) after a thorough mixing of the composite sample taken, pulsified and washed in 2L of distilled water, and then sieved into a container of about 4L.

3.4.3. Salad food: Each salad food sample was weighed and thoroughly washed with 2 L of sterile distilled water into pre-labeled sterile containers (4L).

3.5. MODIFIED EPA METHOD FOR THE IDENTIFICATION OF HELMINTHS EGGS

Helminths eggs were enumerated using a combination of the floatation and sedimentation method (Schwartzbrod, 1998). Samples of water were collected into a 2-L container and allowed to stand overnight to enable the eggs to settle completely. As much of the supernatant as possible was sucked and the sediment transferred into eight 50-ml centrifuge tubes. The 2-L containers were rinsed two to three times with sterile 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 poured away and the deposit was re-suspended in about 150 ml $ZnSO_4$ solution (specific gravity = 1.3) to cause the helminths eggs to float leaving other sediments at the bottom of the centrifuge tube. The mixture was homogenized with a sterile spatula and centrifuged again at 1500 rpm for three min. The $ZnSO_4$ supernatant (containing the eggs) was poured back into the 2-L container and diluted with at least 1L of distilled water, this is to disrupt the specific gravity created by the $ZnSO_4$. This was also allowed to stand for at least three hours for the eggs to settle again. As much supernatant as possible was sucked and deposit was then transferred into 50ml centrifuge tubes. The 2-L container was rinsed two to three times with sterile water and the rinsed water added to the centrifuge tubes and centrifuged at 1500 rpm for three min. The deposits were regrouped into one centrifuge tube and centrifuged at 1500 rpm for three 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

three 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 approximately 1 ml of deposit which was examined under the microscope. The helminths eggs were identified on the basis of their shape and size and compared with the help bench aids for the Diagnosis of Intestinal Parasites (WHO, 1994). The counting was done under a light microscope in both chambers of a haemocytometer at X40 magnification. Viable eggs of helminths especially *A. lumbricoisdes* was determined by their morphology .

3.6 RECOVERY RATE OF THE MODIFIED EPA PROTOCOL

This protocol makes use of the flotation and sedimentation procedures. For flotation with Zinc sulphate (SG 1.3), studies have shown that it has an overall recovery rate of 38.6% of helminth eggs. In comparison with other solutions for flotation, where the top 1.5 ml of the solution is examined, Zinc sulphate (SG 1.3), recovers 94.1%. Higher than the other solutions, which were, sodium chloride (SG 1.2), sucrose solution (SG 1.2) and zinc sulphate (SG 1.2). It is worth noting that in the study by Hawksworth *et al* (2010) which gave the recovery rates quoted above, used seeded soil and feacal matter with *Ascaris* eggs from dissected worms. During microscopy they acknowledge that the eggs were seen to aggregate which they attributed to residues of the uterine wall of the worms. This aggregation of the *Ascaris* eggs could therefore have affected their recovery. Then also in this current study environmental samples were used therefore the aggregation due to uterine wall might be absent or very minimal. Water samples were also not

considered in arriving at the recovery rates mentioned above. The final sedimentation with acetic acid and ethyl acetate would further improve the recovery rate of the helminth eggs.

3.7 HEALTH RISK ASSESSMENT

Quantitative microbial risk assessment (QMRA) approach was used in the health risk assessment. According to Haas *et al.,* (1999) QMRA involves a sequence of interrelated steps: a) hazard identification; b) exposure assessment; c) dose-response assessment and d) risk characterization. QMRA has been used widely in assessing the health risk associated with wastewater use in agriculture (WHO, 2006). The different steps are presented as follows:

3.7.1. Hazard Identification

In this study *Ascaris lumbricoides* was chosen as the main hazard for the risk assessment for farmers and consumers. Several studies have shown a close relationship between *Ascaris* infection and wastewater irrigation (Cifuentes, 1998, Peasey, 2000, Blumenthal *et al.*, 2001). *A. lumbricoides* can survive for long periods of time under severe adverse environmental conditions (Feachem *et al.,* 1983) and has therefore been suggested for QMRAs in developing countries by the WHO (2006). Probability distribution functions (PDFs) were fitted to the concentration of *A. lumbricoides* in the irrigation water and soil using Maximum Likelihood Estimation (MLE) methods. This was to account for the variability and uncertainty. The best PDF that described the data was determined by assessing the Log Likelihood and the Akaike Information Criteria (AIC).

3.7.2. Exposure assessment

Exposure assessment involves the determination of the "amount or number of organisms that correspond to a single exposure (termed the dose) or the total number of *A. lumbricoides* that will constitute a set of exposures" (Haas *et al*., 1999). In this study, four pathways were assessed: (a) Accidental ingestion of only wastewater by farmers; (b) Accidental ingestion of only contaminated soil by farmers; (c) Accidental ingestion of both wastewater and contaminated soil by farmers; and (d) Consumption of salad foods by consumers. The bases for these scenarios are presented in Section 3.7.2.1 and 3.7.2.2.

3.7.2.1. Farmers exposure scenario

Irrigated vegetable farming is a labour intensive exercise, exposing farmers to the wastewater used for irrigation as well as contaminated soil. Since most of these farmers do not wear protective clothes (e.g. boots, mouth covers, gloves etc), they are exposed to pathogens in water and soil. This study accounted for the seasonal exposure of farmers to irrigation water and/or contaminated soil for the wet and dry seasons. The major wet season lasts from March to July, and the minor from September to November, making 8 months in the wet season and 4 in the dry season. If a farmer spends 3 to 5 days on the farm in the wet season then the total number of days spent would be between 96-160 days. And if he spends between 4 to 5 days on the farm in the dry season then the total number of days spent would be between 64-80 days. Therefore all year round the farmer would spend between 160 to 240 days on the farm. These figures were arrived at through a farm observation survey conducted in both seasons. The duration of exposure (i.e

number of days) to *A. lumbricoides* in each season was described with uniform PDF to account for variability and uncertainty.

3.7.2.2. Consumers exposure scenario

Following the production-consumer pathway, most of the vegetables grown in Kumasi were purchased on farm by market women who then take it to the major marketing sites in the city, from where retailers buy from them. Almost all food salads sold within Kumasi are from the farms irrigated with wastewater. A comprehensive study was undertaken to assess the amount of salad consumed in the Kumasi Metropolitan Area (KMA). From this study, the average amount of salad consumed was 40.20g (±8.04) per consumer per day. The frequency of consumption was taken to be four times a week (IWMI 2006; Oboubie *et al*, 2006) and the levels of helminth pathogens in the salad used to determine the amount of pathogens ingested was determined by this study. Probability distribution functions were fitted to the food salad data using MLE methods to account for the variability and uncertainty in the amount of food salad consumed. The best PDF that described the data was determined by assessing the Log Likelihood and the Akaike Information Ctriteria (AIC).

3.7.3. Dose-response assessment

Dose response assessment was undertaken to assess the relationship between the dose of *A. lumbricoides* ingested by farmers and consumers and the probability of infection. In this study, the beta-Poison dose response model developed by Navarro *et al.,(*2009) for assessing *A. lumbricoides* infection was used.

3.7.3.1. Beta-poison dose response model

The Beta-Poison model takes into account the variations which exist in pathogen-host interactions, the parameters for this model were arrived at using the MLE method. A dose response model is acceptable when $Y_{min}$is less than the tabulated chi-square valueχ^2 at k-j degrees of freedom (Haas *et al*., 2000), the beta-poison model developed by Navarro *et al*.,(2009) satisfies this criteria, where $Y_{min}$was 5.074, χ^2 =33. 924, N_{50} of 35, α =0.104 and β=0.044. The probability of infection was therefore calculated based on the following beta-poison model;

$$p(d) = 1 - (1 + \left(\frac{d}{859}\right)\left(2^{\frac{1}{0.104}} - 1\right))^{-0.104}$$

With p(d) being the risk of infection, and *d* the total number of *A. lumbricoides* in a known consumed amount of irrigation water or soil.

3.7.4. Risk characterization

In the risk characterization all the outcomes of the hazard identification, exposure assessment and dose response assessment were combined to characterize the *A. lumbricoides* infection for farmers and consumers. For farmers, the risk characterization was done separately for the wet and dry seasons to account for seasonal variation in the *A. lumbricoides* infection risk.

The risk of infection (P_1(A)) associated with multiple exposures was determined using the formular:

$$P_1(A)=1-(1-P_1(d))^n$$

Where P_1 (d) is the risk of infection from a single exposure to a dose ***d***of *A. lumbricoides*; and ***n*** being the number of days of exposure to the single dose ***d***(Sakaji andFunamizu 1998). For the scenario of farmers' ingesting both irrigation water and contaminated soil, the combined annual risk of infection was determined by using the formular:

$$\Pi t = 1 - (1 - \Pi i)(1 - \Pi x)$$

Where $\mathbf{\Pi_t}$is the combined annual risk of infection from exposures to irrigation water and contaminated soil; $\mathbf{\Pi_i}$ is the *A. lumbricoides* infection risk resulting from accidental ingestion of irrigation water and $\mathbf{\Pi_x}$ is the *A. lumbricoides* infection risk resulting from accidental ingestion of contaminated soil (Haas *et al*. 1999).

Monte Carlo simulation with 10,000 iterations was implemented to account for uncertainty and variability in the risk estimates. All the risk assessment was carried out in R (http://www.r-project.org/).

CHAPTER FOUR

RESULTS

4.1: Occurrence of helminth eggs in farm soil and irrigation water

The concentration of helminth eggs in the contaminated farm soil was more than irrigation water except for *Schistosoma spp*, which was not identified in the soil. The mean concentrations of *A. lumbricoides*, hookworm, *T. trichuira* and *Teania spp* in the soil were 2.77 (±2.12) eggs g^{-1} , 1.61 (±1.53) eggs g^{-1}, 0.37(±0.71)eggs $g^{-1}$and 0.10 (±0.30) eggs g^{-1} respectively. In irrigation water the mean concentrations of *A. lumbricoides*, hookworm, *T. trichuira*, *Taenia spp* and *Schistosoma spp* were 2.11 X 10^{-3} (±1.53)eggs /ml, 0.74 X 10^{-3}(± 0.98) eggs/ml, 0.06 X 10^{-3} (± 0.24) eggs/ml, 0.10 X 10^{-3}(±0.35) eggs/ml and 0.25 X 10^{-3}(±0.58) eggs/ml respectively (Fig. 2).

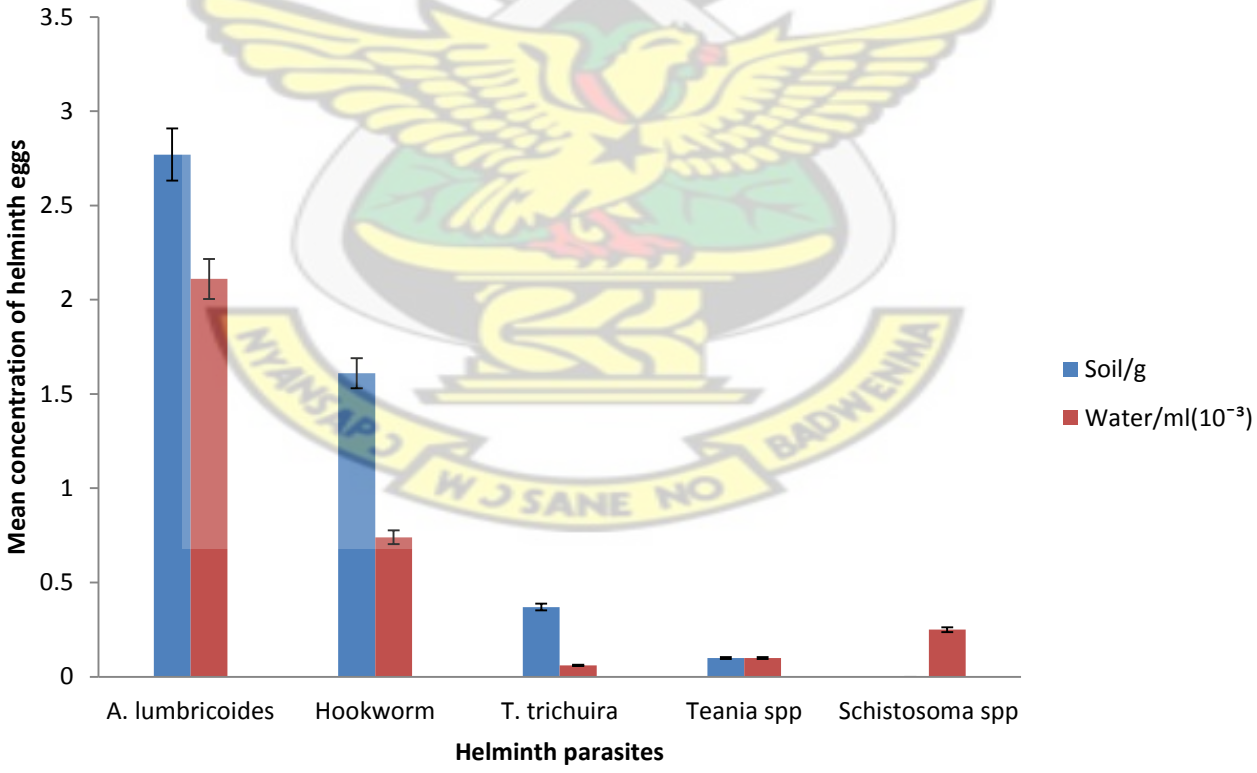

Figure 2: Comparison of mean concentration of helminth eggs in soil and irrigation water.

4.2:Occurrence and seasonal variation of helminth parasites in farm soil

Four parasitic eggs/larvae were identified in the soil samples during the dry and wet seasons. These were *Ascaris lumbricoides*, Hookworm, *Trichuris trichuira* and *Taenia spp*. There was a seasonal variation in the occurrence of helminth parasites in the farm soil. It was more likely to find all the parasites in the farm soils in the wet season than in the dry season. The most prevalent parasite in the wet season was *A. lumbricoides* (87.01%), followed by hookworm (83.12%), *T. trichuira* (25.97%) and *Taenia spp* (10.39%) respectively. The mean concentration of *A. lumbricoides* in soil was however high in the dry season (2.82 eggs g^{-1}) than in the wet season (2.69 eggs g^{-1}). Hookworm and *T. trichuira* had higher mean concentrations in the wet season compared to the dry season (Fig. 3). The seasonal variations was significant for hookworm (p= <0.0001) but insignificant for *A. lumbricoides* (p=0.747), *T. trichuira* (p= 0.863) and *Taenia spp* (p= 0.981).

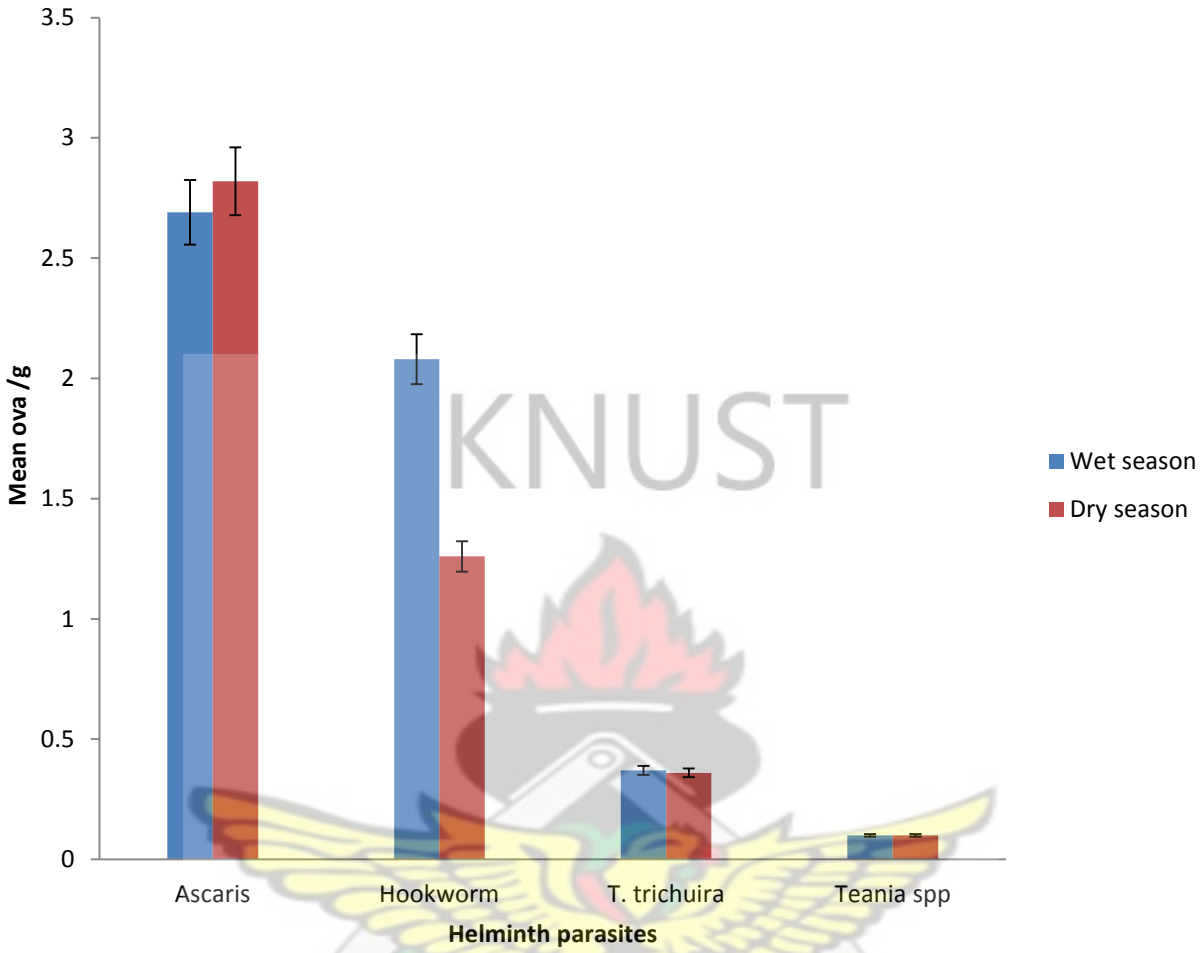

FIG. 3: Mean concentrations of helminth parasites in soil

4.3 Occurrence and seasonal variation of helminth parasites in irrigation water

Five helminth eggs/larvae were identified in the irrigation water. In addition to the four helminths reported for farm soil, *Schistosoma spp* ova were identified in the water samples. The concentration of these parasites in the irrigation water across the study farm sites are presented in Figure 4. As in the soil, the occurrence of *A .lumbricoides* was also relatively high in the irrigation water compared to the other helminth parasites. The mean concentration of *A .lumbricoides*, hookworm, *T. trichuira*, *Taenia spp* and *Schistosoma spp* in the irrigation water

across the farm sites were 2.17 (±1.53) eggs L^{-1}, 0.74 (±0.98) eggs L^{-1}, 0.06 (±0.24) eggs L^{-1}, 0.09(±0.35) eggs L^{-1} and 0.24 (±0.58) eggs L^{-1} respectively. All the parasites identified, except *A. lumbricoides* were more likely to be found in the irrigation water in the wet season compared to the dry season (Figure 4). This seasonal variation in prevalence was significant for hookworm (p = < 0.0001), *T. trichuira* (p = 0.035), *Taenia spp* (p = 0.0022) and *Schistosoma spp* (p = <0.0001) but not significant for *A. lumbricoides* (p=0.919). In terms of seasonal variation, the mean concentrations of *A. lumbricoides* (2.11 eggs L^{-1}) and hookworm (0.44 eggs L^{-1}) were higher in the dry season than the wet season (2.10 eggs and 1.15 eggs L^{-1}) respectively. The vice versa was the case for the remaining helminth parasites (Figure 4).

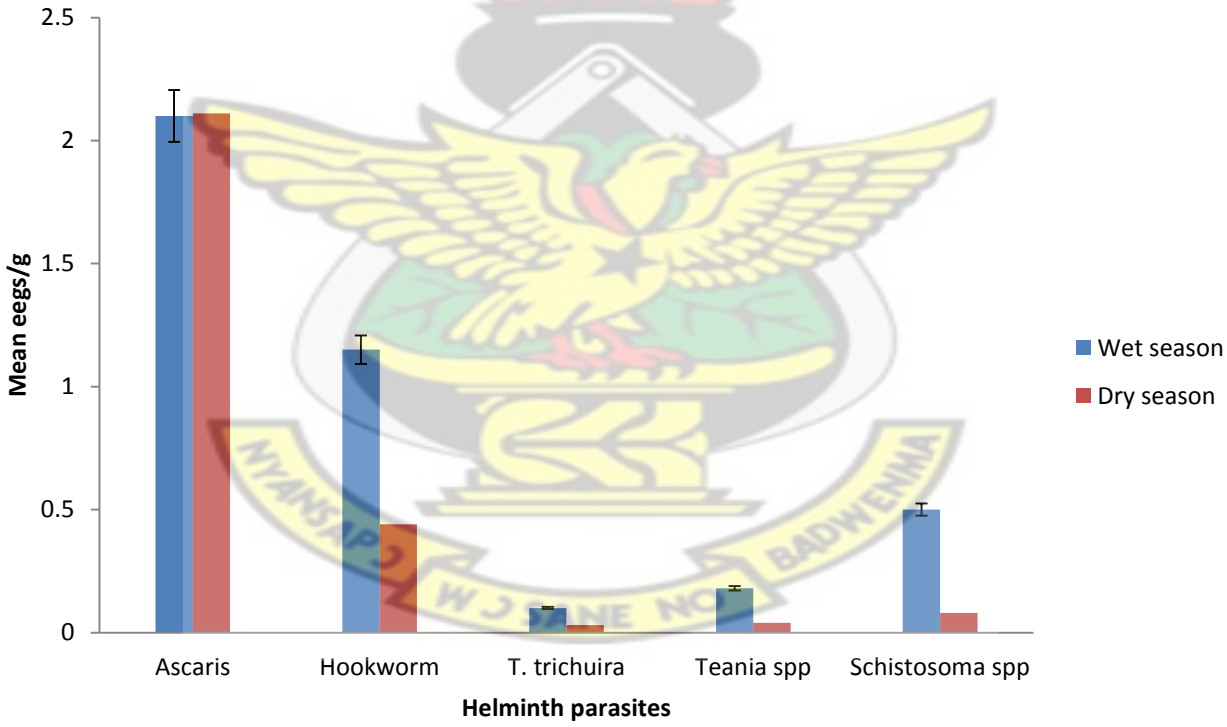

FIG. 4: Occurrence and seasonal variation of helminth parasites in the irrigation water sources.

The mean concentration of helminths in the storm drain, stream, shallow well and pipe waters were 5.25 (±1.28) eggs L^{-1}, 3.92 (±2.09) eggs L^{-1}, 2.34 (±1.64) eggs L^{-1} and 0.5(±0.84) eggs L^{-1} respectively. There was a seasonal variation in the mean concentration of helminths in the different irrigation water sources. The mean concentration of helminths in storm drain, stream, shallow well and pipe irrigation water were 7.00 (±1.53) eggs L^{-1}, 4.83 (±1.85) eggs L^{-1}, 3.06 (±1.34) eggs L^{-1} and 1.0 (±1.0) eggs L^{-1} respectively in the wet season. In the dry season, the mean concentration of helminth parasites in storm drain, stream, shallow well and pipe irrigation water were 4.20 (±0.84) eggs L^{-1}, 3.33 (±2.09) eggs L^{-1}, 1.74 (±1.63) eggs L^{-1} and 0 eggs L^{-1} respectively (Figure 5). There was a significant variation in the occurrence of helminth parasites for the different irrigation waters in the wet season (p= 0.016). However, this variation was not significant in the dry season (p= 0.28).

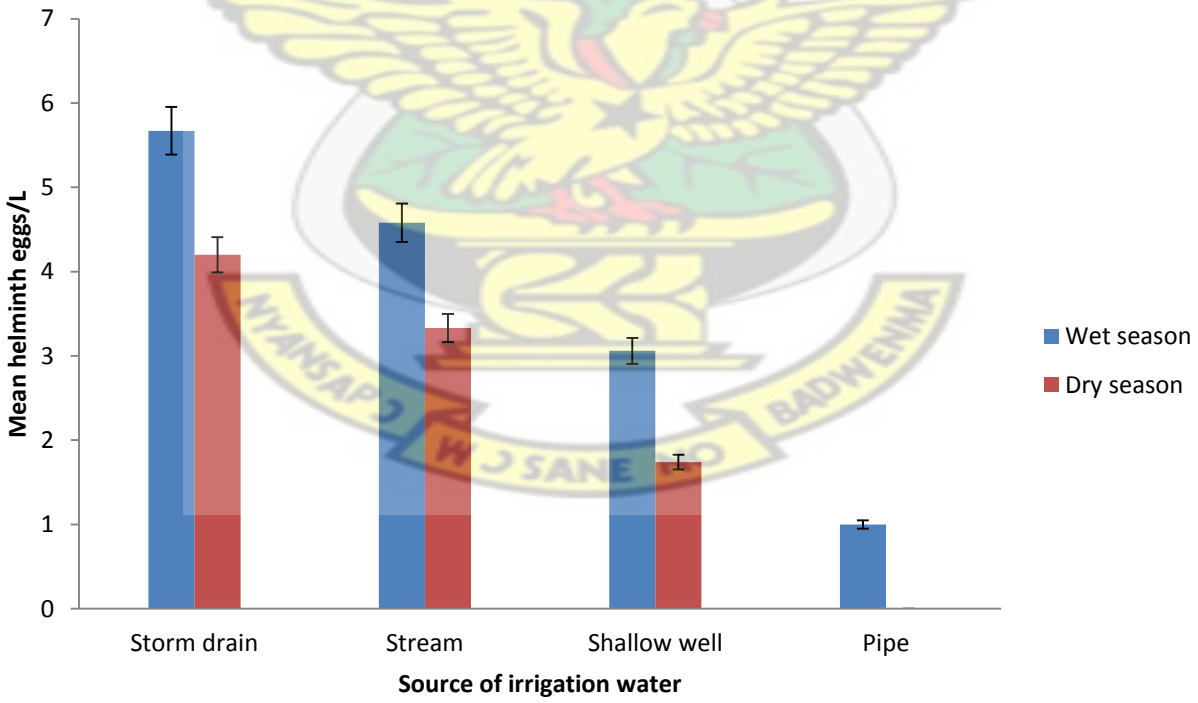

FIG. 5: Mean concentration of helminth eggs/larvae in the different types of irrigation waters.

4.5 Occurrence of helminths in salad food samples

A total of 270 salad samples were analyzed. The prevalence rate of contamination was 7.04% with a mean concentration of 0.13 eggs g^{-1} of salad. Only two parasitic ova were identified, *Ascaris lumbricoides* and Hookworm with means of 0.081(±0.42) eggs g^{-1} and 0.048 (±0.29) eggs g^{-1} respectively (Fig. 6).

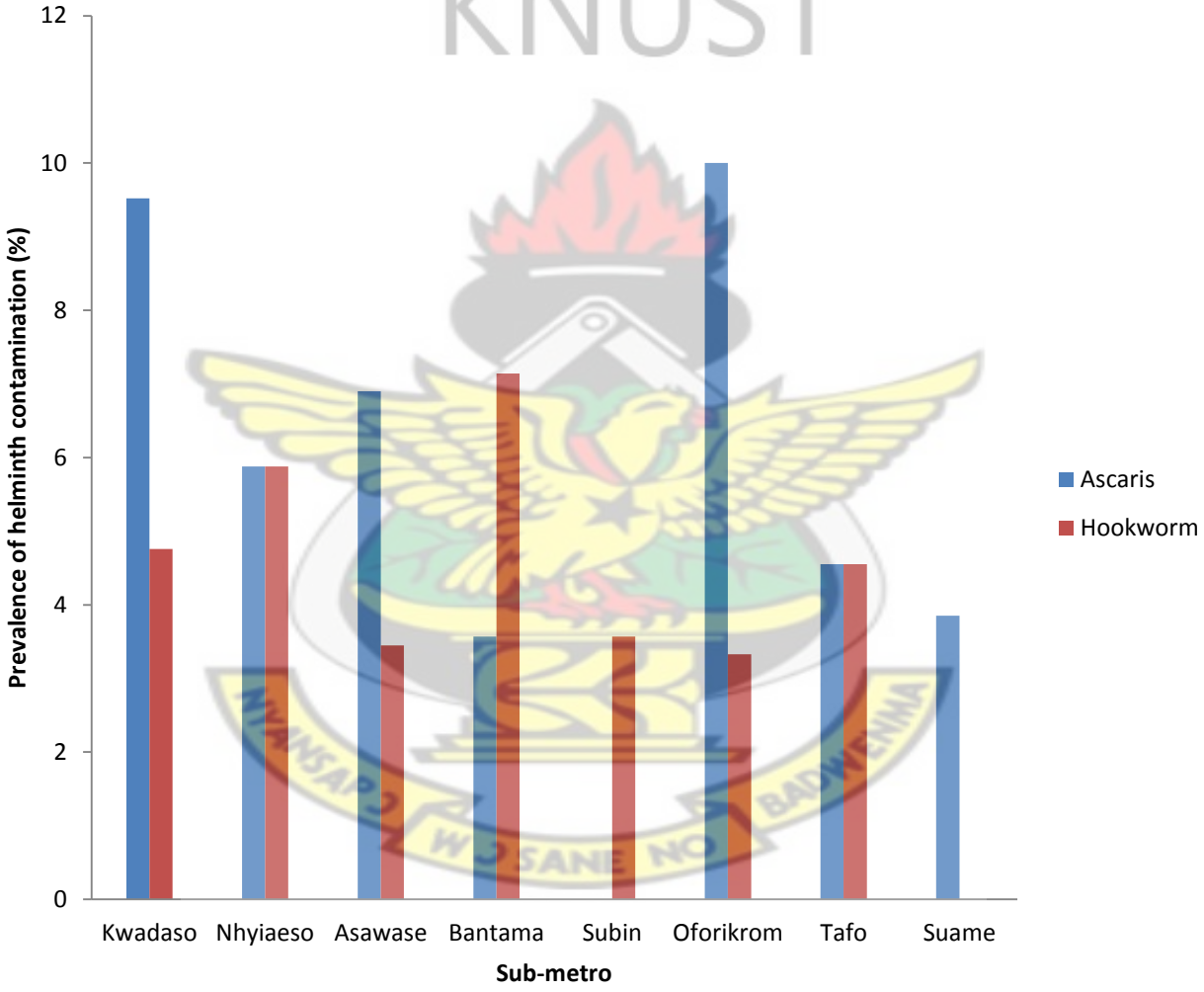

FIG. 6: Prevalence of helminth parasite contamination salad samples from the indicated sub-metros in the Kumasi Metropolis.

4.6: ASCARIS INFECTION RISK TO FARMERS

4.6.1: Uncertainty and variability in the occurrence of *A. lumbricoides* in the exposure pathways

Figures 7-9 show the best fit probability distribution functions (PDFs) and their corresponding bootstraps parameters for *A. lumbricoides* for the different exposure pathways in the wet and dry seasons. These distributions were selected for the risk assessment among several PDFs (See Appendix II). The PDFs and related bootstrapping describe the variability and uncertainty in *A. lumbricoides* in the soil and irrigation water farmers were exposed in the wet and dry seasons.

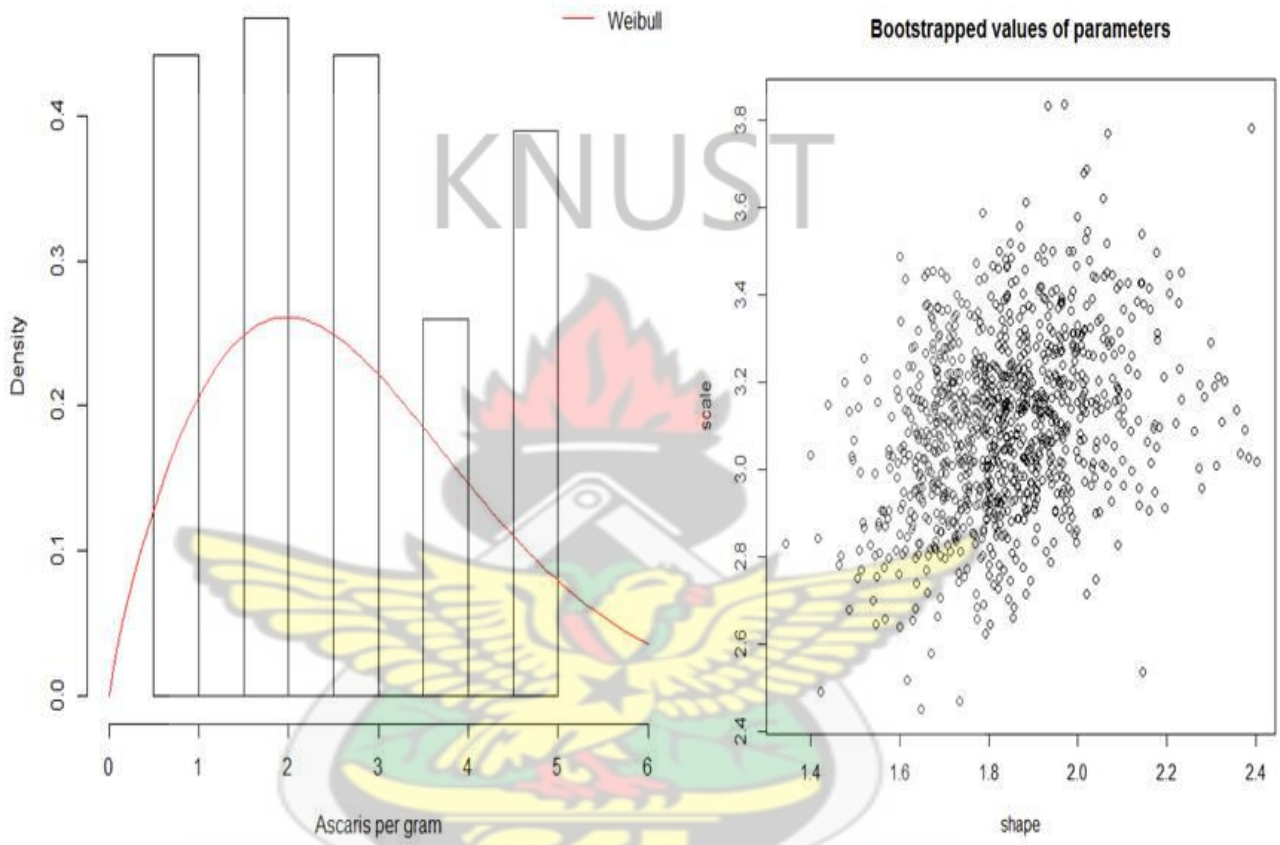

FIG. 7: Concentration of *A. lumbricoides* in the soil in the dry season as described by Weibull PDF (left) and corresponding bootstrapped values of the shape and scale parameters of the distribution (right).

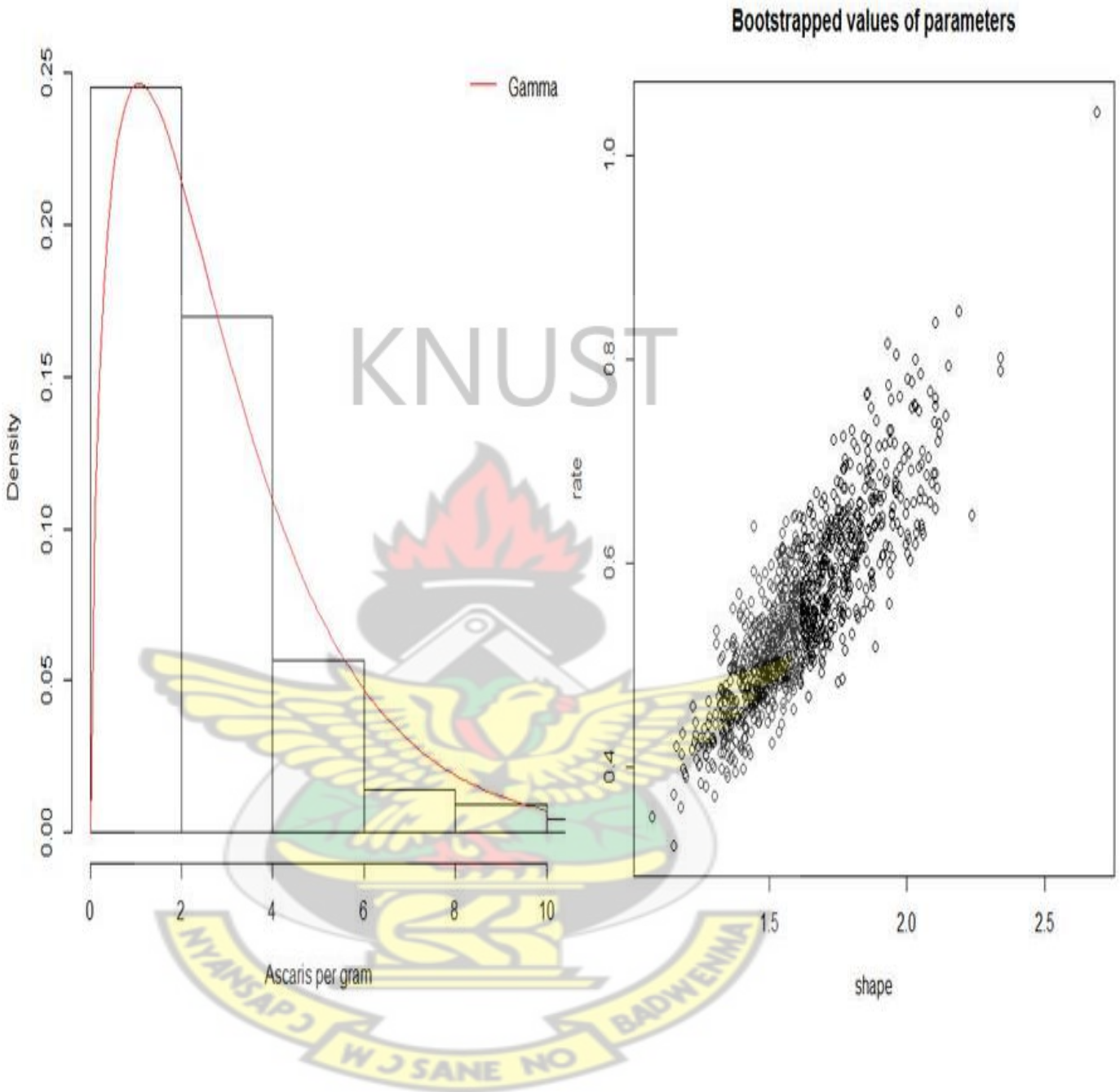

FIG. 8: Concentration of *A. lumbricoides* in the soil in the wet season as described by Gamma PDF (left) and corresponding bootstrapped values of the shape and rate parameters of the distribution (right).

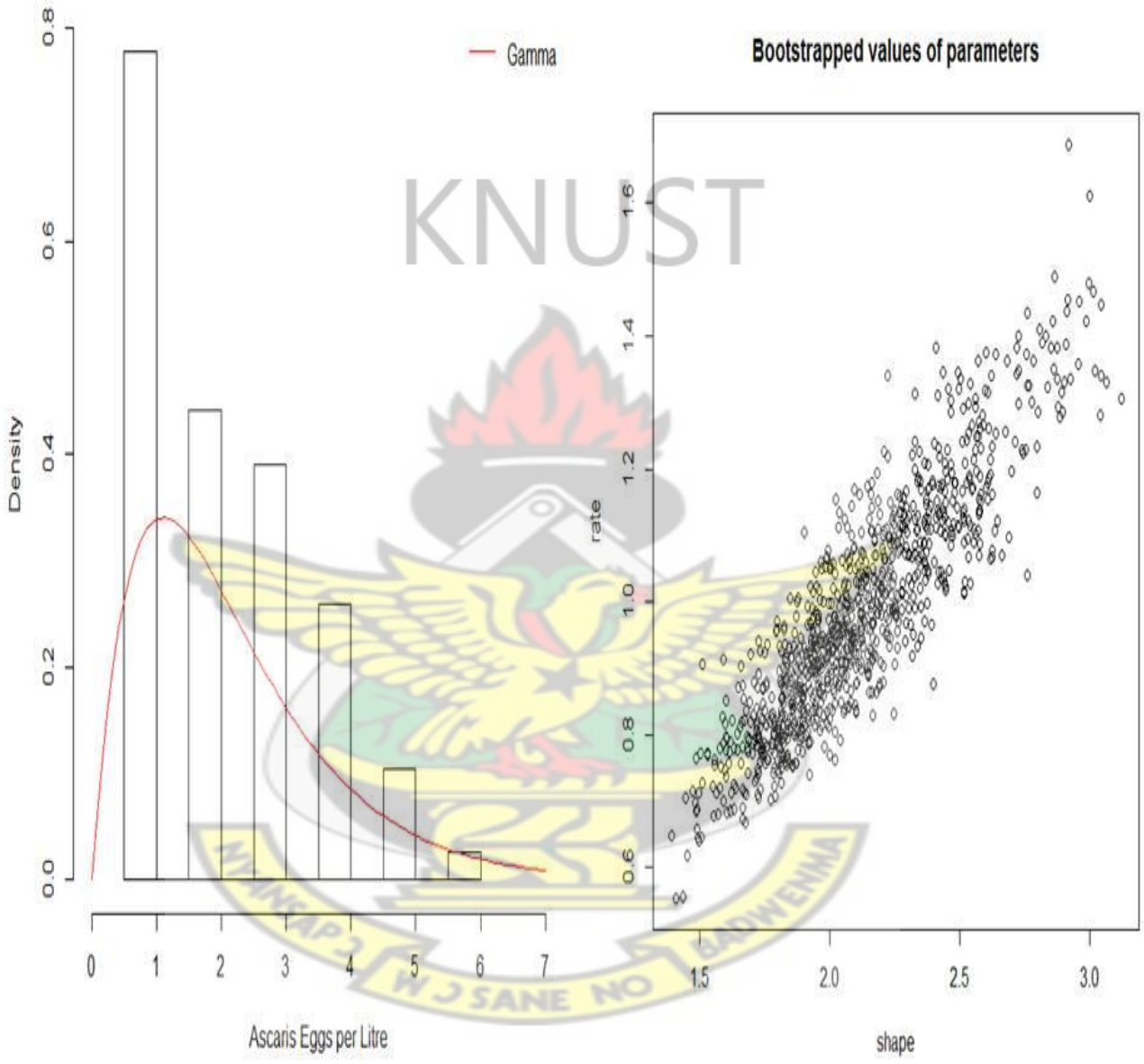

FIG. 9: Concentration of *A. lumbricoides* in irrigation water in the dry season as described by Gamma PDF (left) and the corresponding bootstrapped values of the shape and rate parameters of the distribution (right).

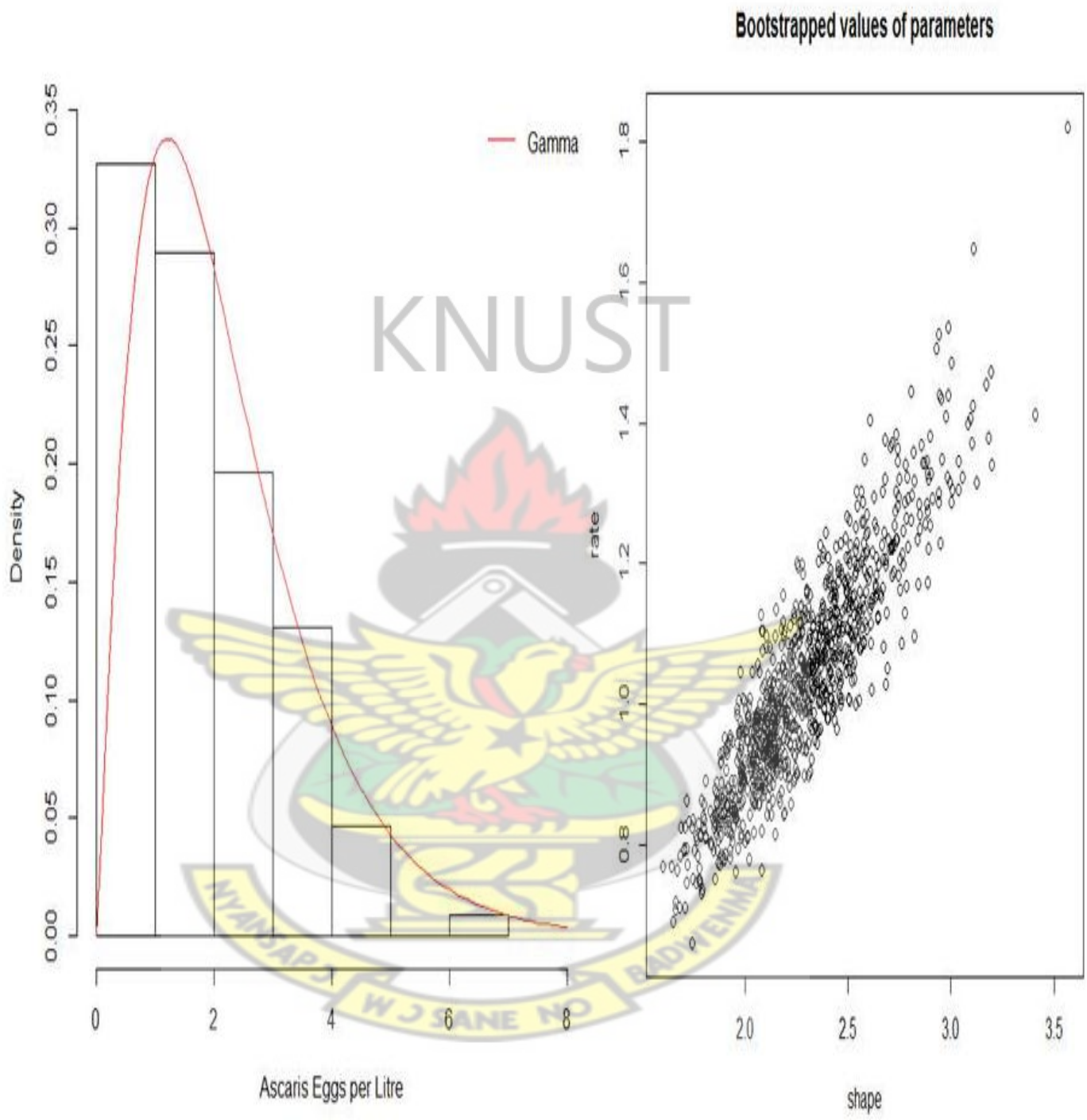

FIG. 10: Concentration of *A. lumbricoides* in irrigation water in the wet season as described by Gamma PDF (left) and corresponding bootstrapped values of the shape and rate parameters of the distribution (right).

TABLE 4.2: Summary of best fit PDFs and bootstrapped parameters

Exposure Pathway	Best fit PDF	Parameters	Bootstrapped values of parameters (95%CI)	Log-Likelihood	AIC
Soil (dry season)	Weibull distribution	Shape=1.827 Scale=3.105	Shape=1.54-2.19 Scale=2.7-3.48	-139.93	283
Soil (wet season)	Gamma distribution	Shape=1.594 Rate=0.544	Shape=1.26-2.05 Rate=0.41-0.74	-214.28	432
Irrigation water (dry season)	Gamma distribution	Shape=2.068 Rate=0.957	Shape=1.57-2.86 Rate=0.71-1.36	-127.20	258
Irrigation water (wet season)	Gamma distribution	Shape=2.242 Rate=1.007	Shape=1.76-2.94 Rate= 0.77-1.36	-176.57	357

4.6.2: *A. lumbricoides* infection to farmers associated with seasonal exposure to soil or irrigation water only

Farmers exposed to soil on farm in the wet season were more likely to be infected with *A. lumbricoides* 6.5 $x10^{-1}$ per farmer (95% CI: 0.609-0.695) than in the dry season, 5.27 x 10^{-1} per farmer (95% CI: 0.484-0.567). A similar seasonal pattern of *A.lumbricoides* infection risk was associated with exposure to irrigation water. Farmers exposed to irrigation water in the wet season were more likely to be infected with *A. lumbricoides*, 7.5x 10^{-2} per farmer (95%CI: 0.066-0.085), compared with the dry season, 4.3x 10^{-2} per farmer (95% CI: 0.037-0.050). The cumulative probability distributions of the *A. lumbricoides* infection risks for exposure pathway to soil and irrigation water are presented in Figure 11.

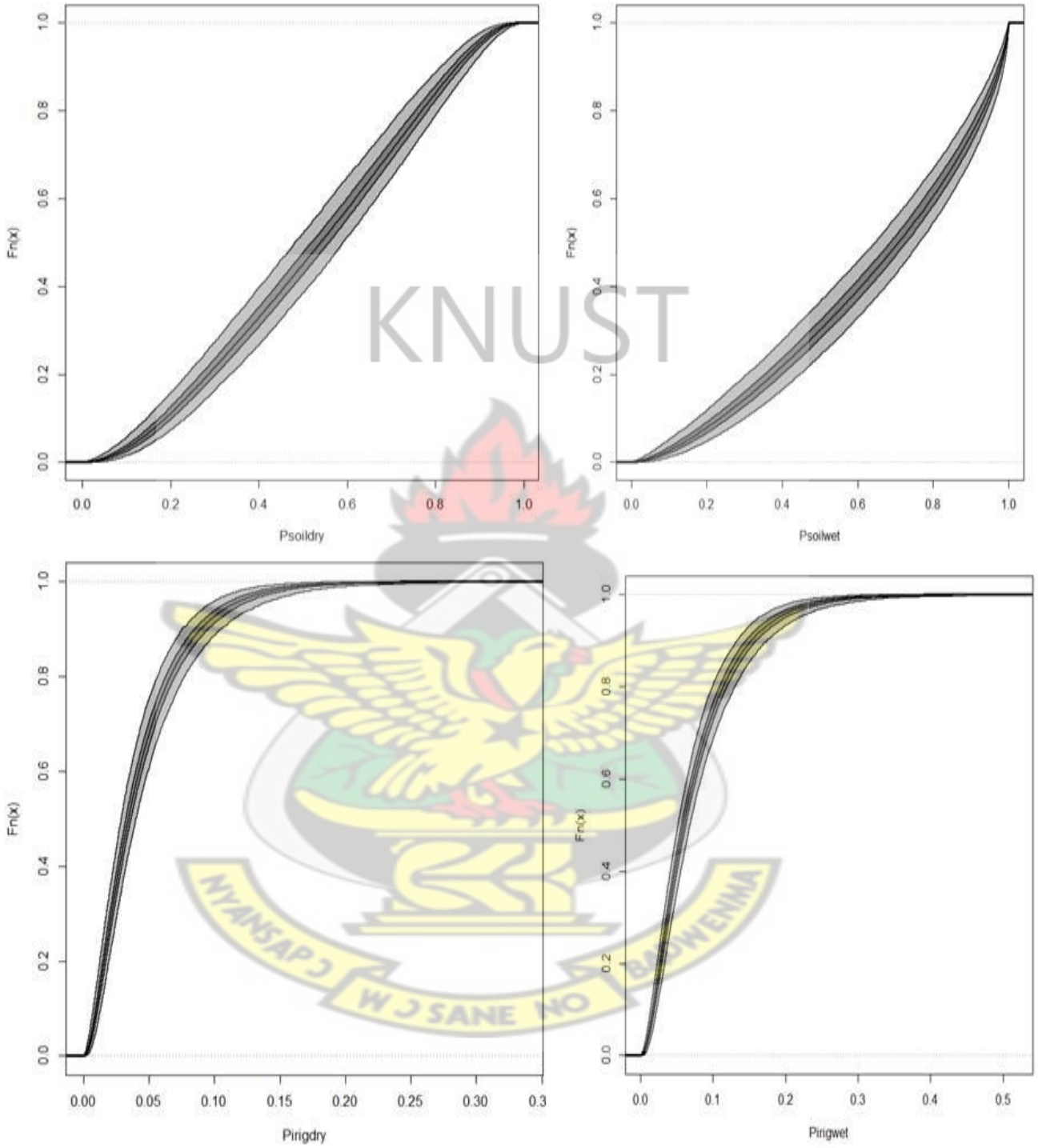

FIG: 4.11: **Cumulative probability distribution of the *A. lumbricoides*, infection associated with exposure to soil in the wet season (Psoilwet); soil in the dry season (Psoidry); irrigation water in the dry season (Pirrigdry) and irrigation water in the wet season (Pirrigwet).**

4.6.3: *A. lumbricoides*infection risk associated with exposure to both soil and irrigation water

The mean *Ascaris* infection risk to farmers exposed to both soil and irrigation water in the wet season was 6.8 x 10^{-1} per farmer (95% CI 0.63-0.72), compared with the infection risk associated with the same exposure pathway in the dry season5.5 x10^{-1} per farmer (95% CI: 0.51-0.58) (Fig 4.11). Irrespective of season, the annual infection risk associated with exposure to irrigation water and soil was 0.85 x 10^{-1} per farmer per year (95%CI: 0.83-0.87) (Fig. 4.12).

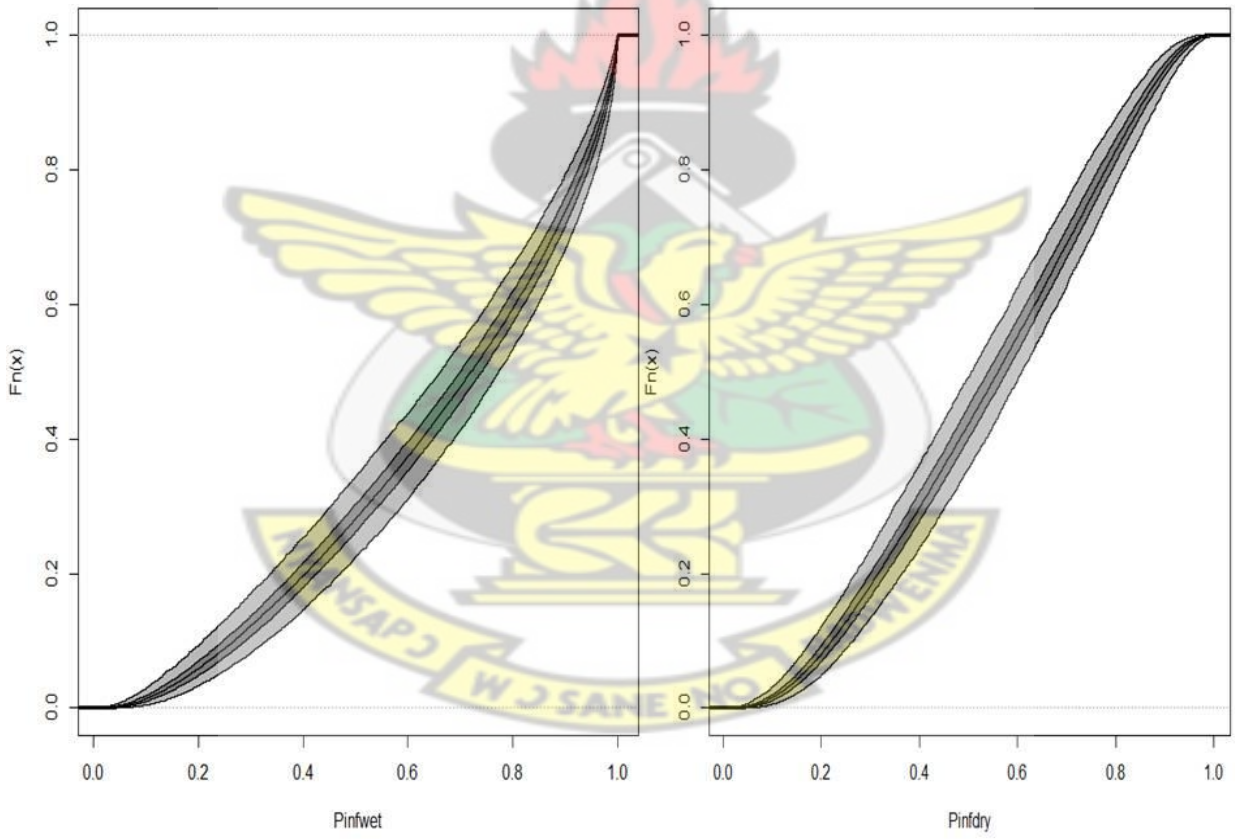

FIG. 12:Cummulative probability distribution of the *A. lumbricoides* infection risk in the wet (Pinfwet) and dry (Pinfdry) seasons associated with exposure to both soil and irrigation water

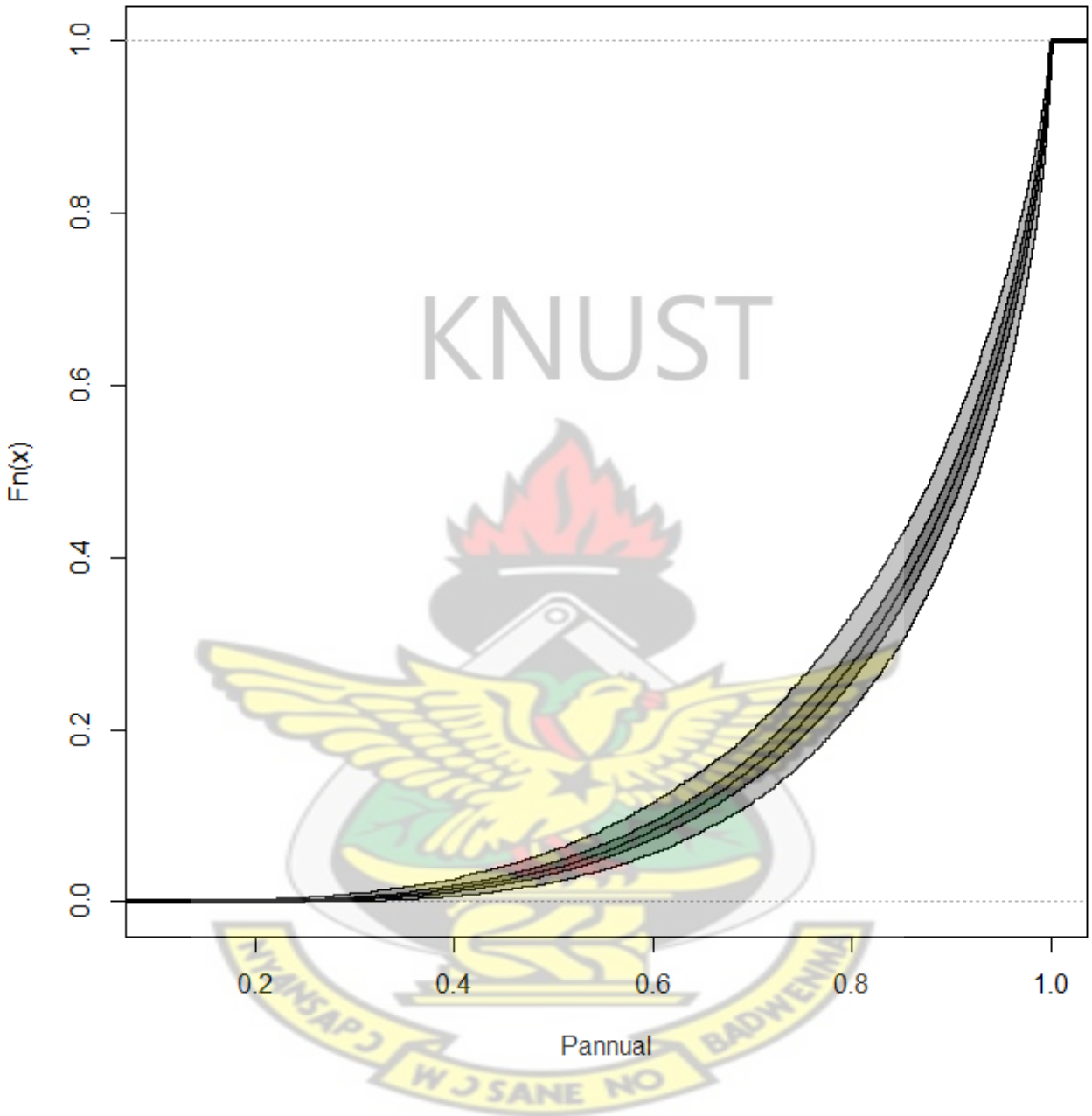

FIG. 13: **Annual risk of infection with *A. lumbricoides*for exposure to both soil and irrigation water (pannual)**

4.7: ASCARIS INFECTION RISK TO CONSUMERS

4.7.1: Uncertainty and variability of the amount of salad consumed and the occurrence of *A. lumbricoides* in salad foods

The best fit PDF for the amount of salad consumed was also best described by the Lognormal distribution (meanlog=3.6; sdlog=0.1).The best fit PDF for *A. lumbricoides* in salad was also the lognormal distribution (meanlog= -0.65; sdlog=0.23). The PDFs and their corresponding bootstrapped values of their parameters are shown in the Figures 14 and 15 below.

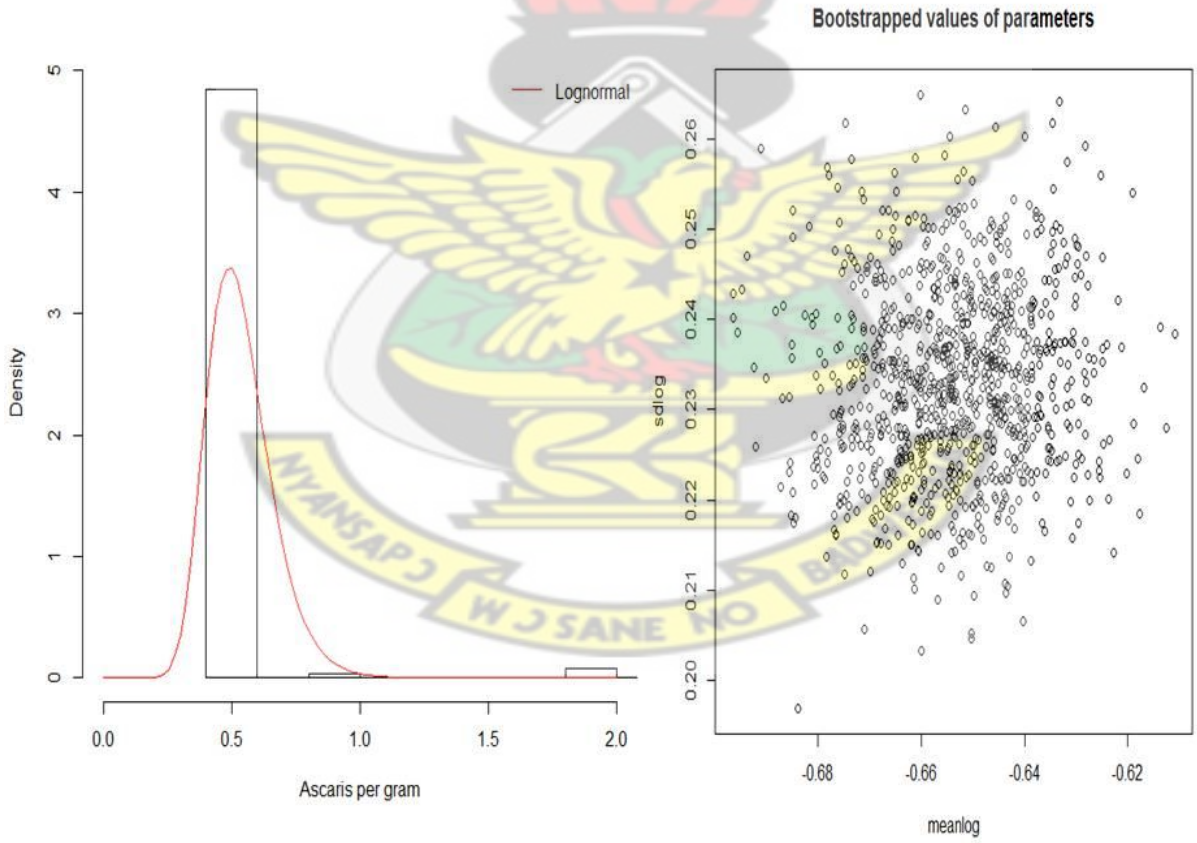

FIG. 14: Concentration of A.lumbricoides in salad foods as described by LogNormal PDF and the corresponding bootstrapped values of the MeanLog and Sdlog values of the distribution.

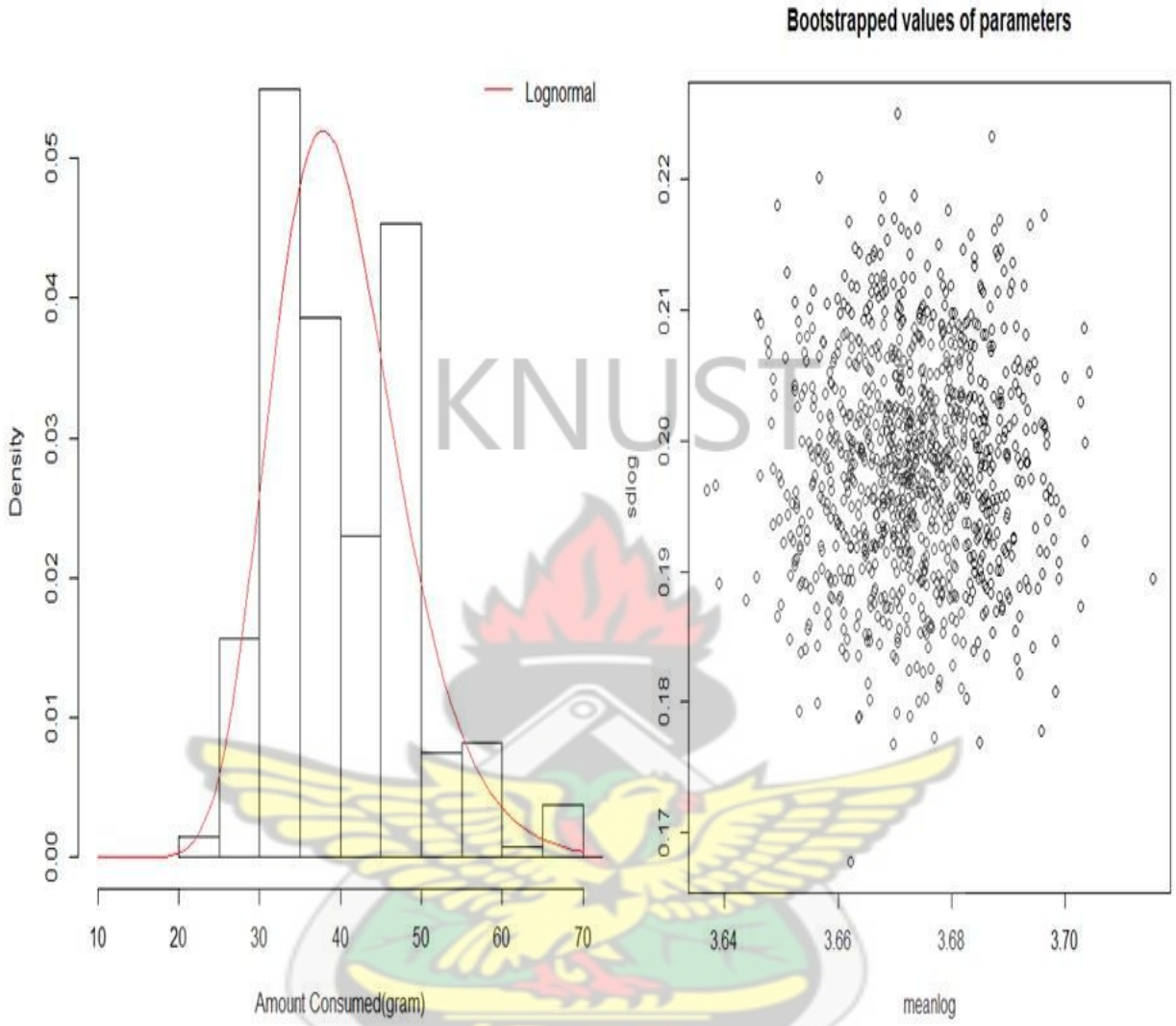

FIG. 15: Amount of salad consumed as fitted with LogNormal distribution and corresponding bootstrapped values of the sdlog and meanlog values of the distribution.

TABLE 4.3: Summary of best fit PDFs and bootstrapped parameters

Exposure parameter	Best fit PDF	Parameters	Bootstrapped values of parameters (95%CI)	Log-Likelihood	AIC
Amount of salad consumed	Lognormal	Meanlog=3.65 Sdlog=0.1	3.60-3.69 0.18-0.21	-934.96	1873.92
Ascaris concentration in salad	Lognormal	Meanlog= -0.65 Sdlog= 0.23	-0.68 – (-0.63) 0.21- 0.25	185.60	-367.21

4.7.2 Ascaris infection risk associated with the consumption of salad

The *A. lumbricoides* infection risk associated with consumption of salad foods is 2.6×10^{-1} per consumer per year (95CI: 0.22-0.30) (Figure 16). This estimate assumes a single event consumption of salad in the streets of the Kumasi Metropolitan Area. If a consumption rate of 156 per year is assumed, then on average, all those consuming salad will be infected with *A. lumbricoides*.

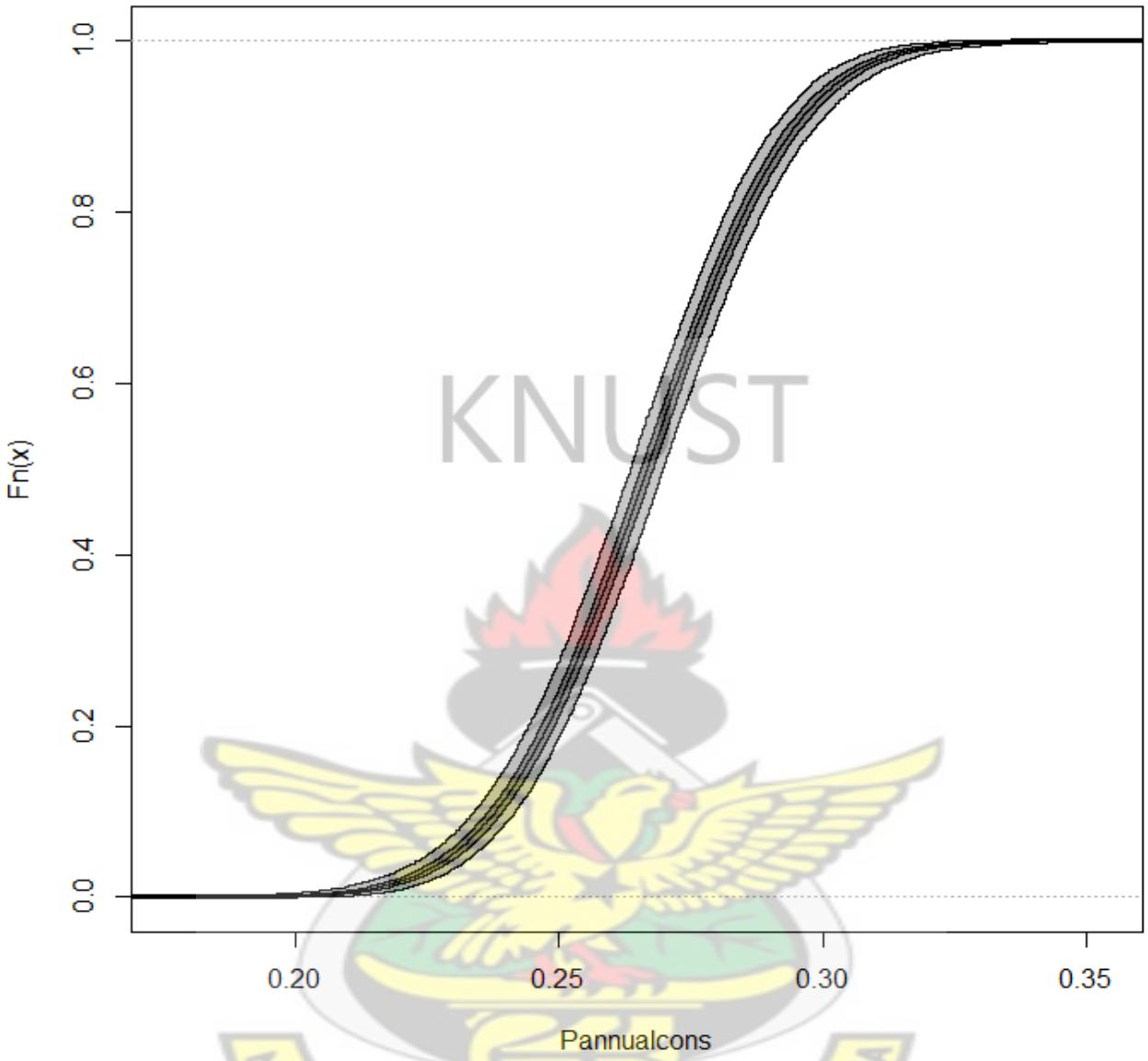

FIG. 16: Annual infection risk associated with the consumption of salad potentially irrigated with wastewater (assumes a one time exposure)

CHAPTER FIVE

DISCUSSION

Helminth concentrations in soil and irrigation water was found to be prevalent in the study area, exceeding the WHO recommended standard of <1 egg L^{-1} for unrestricted irrigation (WHO,2006). Four helminth eggs were identified in farm soil in this study, these were *A. lumbricoides*, hookworm, *T. trichuira* and *Taenia spp*. In addition to these four, *Schistosoma spp* was found in irrigation water. These results confirm those obtained by many workers (Ackerson and Awuah, 2012; Klutse, 2009; Andoh , 2008; Hajjami *et al*., 2013; Klutse and Baleux,1995 and Jimenez *et al*.,2010) .

Out of the four helminth parasites identified in this study, *A. lumbricoides* was the most predominant, confirming the importance of the parasite as a health hazard in wastewater used for irrigation. The predominance of *A. lumbricoides* in the wastewater irrigated farms is consistent with findings made in other studies. Earlier studies in Kumasi reported the dominance of *A. lumbricoides* in wastewater irrigated farms (Amoah *et al*., 2009; Ackerson and Awuah, 2012). Also on vegetable farms in Marrakech, Bouhoum *et al*., (1997), showed that *A. lumbricoides* eggs concentration was high (75.6 eggs L^{-1}) in irrigation water and was the most predominant (52%) of the helminth eggs in the irrigation water used by the farmers. Hajjami *et al.*, (2013); Klutse and Baleux (1995) and Jimenez *et al* (2010) all confirmed the dominance of *A. lumbricoides* in wastewater used for irrigation from studies conducted in Morroco, Sudan and Mexico respectively. Another finding made in this study was the presence of *Schistosoma spp* eggs in the irrigation water used by the farmers. *Schistosoma eggs* are known to hatch into miracidium upon contact with water after release into the environment, this could account for

their absence in the farm soil. This confirms results obtained by Klutse (2009) and Andoh (2008) who also reported of varying concentrations of *Schistosoma spp* in irrigation water.

This study revealed a seasonal variation in the occurrence of helminth in irrigation water and soil. Compared to other helminths, it was more likely to find *A. lumbricoides* in the dry season than in the wet season (Fig. 2). The climatic conditions in Kumasi allow helminth parasites to persist for long time irrespective of the season of the year. The WHO (2006) estimates that at temperatures of 20^0C -30^0C (similar to what exists in Kumasi), *A. lumbricoides* eggs can survive in soil and fresh water and sludge for years, while *Teania spp* can only survive for months. In the wet season, the concentration of *A. lumbricoides* in the farm soil was much lower than in the dry season, this could be attributed to washing by run-off into the water bodies (streams, shallow wells etc).

Farmers used four sources of irrigation water (Amoah *et al.*, 2005) with the exception of storm drains. However in this study five sources of irrigation water were identified; storm drain, shallow well, river/stream and pipe water. Storm drain had the highest mean concentration of helminth eggs (5.25 eggs L^{-1} (±1.28)). The WHO recommends a concentration of <1 helminth egg per liter of irrigation water, the storm drain water exceeded this standard and therefore could be a source of helminth infection for the farmers and consumers of the vegetables. The storm drains are fed by water from water bodies contaminated with wastewater and could be the reason for the presence of helminth eggs in all storm drain samples.

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. These results obtained by earlier studies agree with the results of this study. The river/stream irrigation water

had a mean concentration of 3.92 eggs L^{-1}(±12.09) (Fig. 5), which is similar to reports in the earlier studies mentioned. With the exception of pipe-borne water used for irrigation, all other irrigation sources had mean helminth egg concentrations exceeding the WHO standard for irrigation water. The prevalence of the rest of helminth parasites in the other sources of water showed a seasonal variation. All the sources of irrigation water had at least one helminth egg irrespective of the season, with the exception of pipe-borne water in the dry season. Similar results were reported by Amoah *et al.*,(2005) and Klutse (2009). However in those studies pipe-borne water did not have any contamination with helminth eggs and these studies did not take into account the seasonality. During the wet season runoffs from the farm soil and surrounding fields is expected to be high. This could account for the higher concentration of helminth eggs in the water bodies (Drechsel *et al.,* 2000; Amoah *et al*., 2005). Contamination of pipe water with helminth eggs in the wet season could be due to cracks and leakages in the piping system allowing soil and contaminated water access to the pipe water and in the process contaminating it. There was significant variation (p=0.016) between the mean concentration of the helminth eggs in the various sources of wastewater in the wet season. However, there was no significance (p =0.28) in the dry season.

The annual risk of infection with *A. lumbricoides* (8.5×10^{-2}) is higher than the recommended tolerable risk of infection (1.2×10^{-2}) (Mara and Sleigh, 2010) and one order greater than annual infection risk obtained by Seidu *et al.,* (2008) and Ackerson and Awuah (2012) in Ghana. Cutolo *et al*.,(2012) also obtained infection risks of 7.5×10^{-2} and 8.7×10^{-2} for 208 and 240 days respectively of exposure to wastewater for wastewater irrigation farmers in Brazil. In terms of seasonal infection risk, farmers exposed to both soil and irrigation water were more likely to be infected in the wet season than the dry season but all within the same magnitude (10^{-1}). The same

magnitude of *A. lumbricoides* infection risk (10^{-1}) was found for exposure to only farm soil and only irrigation water for the two seasons. Comparing the risk of infection with *A. lumbricoides* for the farmers due the accidental ingestion of only soil and only irrigation water in the wet and dry seasons indicated that the farmers are at a greater risk of infection due to ingestion of farm soil than irrigation water only. This could be attributed to the high concentration of *A. lumbricoides* eggs in the farm soil due to years of wastewater irrigation. Therefore there is an increase risk of infection with *A. lumbricoides* due to accidental ingestion of soil than there is for ingestion of irrigation water in the dry season. Seidu *et al* (2008) obtained similar results using data from studies carried out in Accra, Ghana.

Helminth egg concentration in salad foods was predictably low (mean concentration of 0.13 g^{-1} and prevalence rate of 7.04%), with *A. lumbricoides* and hookworm being the only types of helminth eggs identified. However *A. lumbricoides* had a higher mean concentration (0.08 g^{-1}) than hookworm (0.048 g^{-1}), probably because of its higher survival rate and prevalence in the wastewater irrigated vegetable farms. Andoh (2008) also reported a similar pattern in helminth contamination, however in that study *A. lumbricoides* and *Schistosoma haematobium* were the helminth eggs identified in salad foods sold in Kumasi. There are various interventions employed by food sellers in Kumasi in an attempt to reduce the concentration of pathogens on the vegetables, some of which have been reported to cause a reduction in these pathogens Amoah *et al.,* (2005). However the extent of their efficiency is limited by the concentration of the solutions used and the duration of the disinfection. It is estimated that there is a 67% reduction in helminth eggs from the farm to the kitchen (Andoh, 2008). This is attributed to eggs being desiccated from exposure to unfavourable environmental conditions (Larkin *et al.,* 1978). The estimated risk of infection with *A. lumbricoides* due to the consumption of salad foods was

estimated to be 2.6 x 10^{-1} per consumer per year, higher than the tolerable risk of infection. Drechsel *et al.,* (2000) reported that it is difficult to find any irrigated (lettuce, spring onion, and cabbage) sold on markets which is not contaminated with helminths. Ulukanligil *et al.,* (2001) started in Turkey nearly half of the vegetables including lettuce irrigated with wastewater and sold on the market were contaminated with *A. lumbricoides*. From this study it can be seen that helminth loads in wastewater used for irrigation is still high resulting in a higher risk of infection with these pathogens for both the farmers and the consumers of vegetables irrigated with wastewater.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study revealed a high helminth egg concentration in irrigation water and farm soil used by vegetable farmers in the Kumasi Metropolitan Area. Irrespective of the season the concentrations of these helminths exceeded the WHO limit for all irrigation water sources except pipe-borne water. The annual risk of infection with *A. lumbricoides* was higher than the tolerable risk of infection for wastewater irrigation. This study also revealed that, vegetable farmers in the study area were at a higher risk of *A. lumbricoides* infection due to accidental ingestion of only farm soil than ingestion of only irrigation water irrespective of the season. Contamination of salad foods with *A. lumbricoides* and hookworm was also recorded. Consumers of vegetables cultivated with wastewater were therefore equally at risk of *A. lumbricoides* infection to the same extent as the farmers ($\times 10^{-1}$).

6.2 RECOMMENDATIONS

With increasing urban population, the demand for limited water resources would also increase leading to an increase in the use of wastewater for irrigation. Therefore to reduce the risk of helminth infections, the following measures are recommended;

1. Practical, simple and inexpensive methods of improving the microbial quality of irrigation water at the farm level be developed or an alternative source of water be provided for irrigation.

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 such as gloves, boots, trousers and long sleeve shirts during farm work to reduce the level of exposure.

4. Education on the right methods for vegetable washing especially at the point of consumption should be increased by the agricultural extension officers.

5. The development of practical local guidelines by the Ministry of Food and Agriculture and then the Ministry of Health that would get the cooperation of all stakeholders towards implementation

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APPENDICES

APPENDIX I: Mean concentration of helminths

Table 4.3: Mean helminth egg concentration in farm soil

Helminths	Dry season			Wet season		
	Mean values	Standard deviation	Range	Mean values	Standard deviation	Range
Ascaris	2.69	1.67	1-5	2.82	2.41	1-12
Hookworm	2.08	1.54	1-5	1.26	1.43	1-7
T. trichuira	0.37	0.70	1-3	0.36	0.72	1-3
Teaniaspp	0.10	0.03	0-1	0.10	0.31	0-1
Schistosomaspp	0	0	0	0	0	0

Table 4.4: Mean helminth egg concentration in irrigation water

Helminths	Dry season			Wet season		
	Mean values	Standard deviation	Range	Mean values	Standard deviation	Range
Ascaris	2.10	1.58	1-6	2.11	1.5	1-7
Hookworm	1.15	1.12	1-5	0.44	0.73	1-3
T. trichuira	0.10	0.31	1-2	0.03	0.17	0-1
Teaniaspp	0.18	0.45	1-2	0.04	0.23	1-2
Schistosomaspp	0.50	0.73	1-3	0.08	0.33	1-2

Table 4.5: Prevalence of helminth eggs/larvae in the different types of irrigation waters.

	DRY SEASON	WET SEASON
	Prevalence (%)	Prevalence (%)
Strom drain	100	100
Stream	100	96.72
Shallow well	93.75	74.36
Pipe	66.67	0

APPENDIX II

Table 4.6: Summary of best fit PDFs and bootstrapped parameters.

Exposure Pathway	**Best fit PDF**	**Parameters**
Soil (dry season)	Weibull distribution	Shape=1.827 Scale=3.105
Soil (wet season)	Gamma distribution	Shape=1.594 Rate=0.544
Irrigation water (dry season)	Gamma distribution	Shape=2.068 Rate=0.957
Irrigation water (wet season)	Gamma distribution	Shape=2.242 Rate=1.007

Table 4.7: Mean risk of infection for the various pathways.

EXPOSURE PATHWAY	**MEAN INFECTION RISK (CI)**
Soil (dry season)	0.527 (0.484-0.567)
Soil (wet season)	0.653 (0.609-0.695)
Irrigation water (dry season)	0.043 (0.037-0.050)
Irrigation water (wet season)	0.075 (0.066-0.085)
Both soil and irrigation water (dry season)	0.977 (0.973-0.981)
Both soil and irrigation water (wet season)	0.957 (0.944-0.958)
Annual risk	0.999 (0.999-0.999)

APPENDIX III

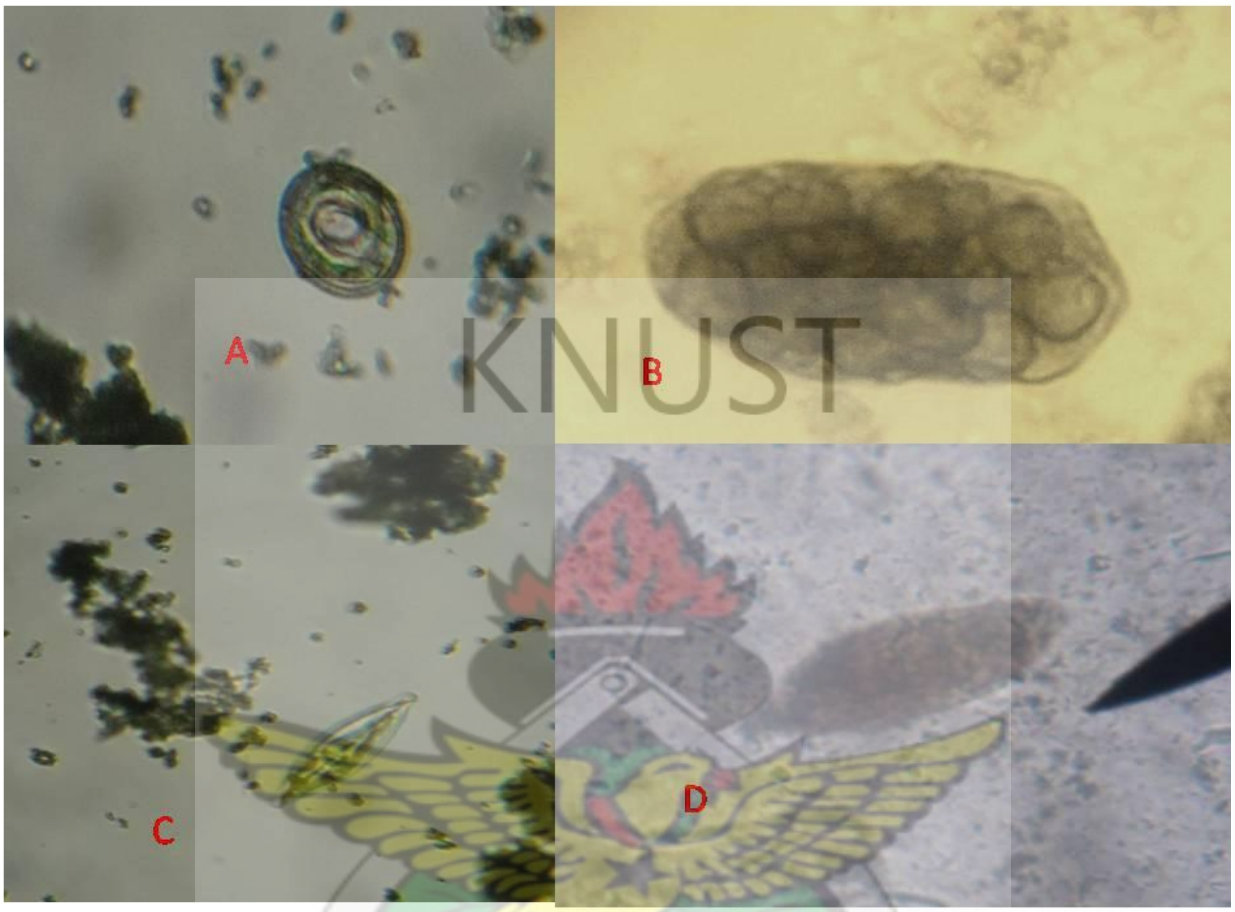

Plate 2: Pictures of *Ascarislumbricoides* (A), hookworm (B), *Trichuristrichuira* (C) an d *Schistosomaspp* (D) as seen under X10 lens of the microscope.