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MICROBIAL ANALYSIS OF SOIL SAMPLES IN A WASTEWATER IRRIGATED VEGETABLE PRODUCTION SITE: CASE STUDY AT ATONSU, KUMASI

BY

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CERTIFICATION

I, KLUTSE COURAGE KWASHIE HEREBY DECLARE THAT THIS SUBMISSION IS MY OWN WORK TOWARDS THE MSc AND THAT, TO THE BEST OF MY KNOWLEDGE, IT CONTAINS NO MATERIAL PREVIOUSLY PUBLISHED BY ANOTHER PERSON NOR MATERIAL WHICH HAS BEEN ACCEPTED FOR THE AWARD OF ANY OTHER DEGREE OF THE UNIVERSITY, EXCEPT WHERE DUE ACKNOWLEDGEMENT HAS BEEN MADE IN THE TEXT.

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ABSTRACT

Though irrigation with wastewater has potential benefits of meeting water requirements as well as providing organic matter and nutrients to soil, it may also pose health hazards to farm workers and consumers of food products from wastewater-irrigated crop production. The soil then becomes a reservoir of enteric pathogens and has the potential to transmit many various diseases of enteric origin. The study was conducted on urban vegetable farming sites at Atonsu in Kumasi, Ghana. The objective of this research was to determine the effect of irrigation with wastewater on the microbiology of soils under vegetable cultivation by, (a) assessing the soil contamination levels in wastewater irrigated agricultural plots in urban Kumasi, and (b) detecting the presence of indicator organisms associated with agricultural plots. Soil samples from two different depths (0 – 30 cm and 30 – 45 cm) were collected from fields treated with different irrigation water sources and analysed. The treatments include plots where: (i) wastewater irrigation practice has been going on for over a decade , (ii) piped-water (PW) irrigation practice has been used for over a decade (control), and (iii) no recent cultivation has taken place (control). The irrigation water sources were also sampled for analysis. Both soil and irrigation water samples were analyzed for different physicochemical (pH, soil moisture, soil texture) and biological parameters (Total coliforms (TC), Fecal coliforms (FC), and helminth eggs) using standard methods. Soil samples from all three plots carried FC and helminth egg populations ranging between 0.03 to 9.5 x 10^4 per 10g for FC and 0 to 30 per 10 g for helminth. A number of different types of helminth eggs, including that of *Ascaris lumbricoides, Strongiloides stercoralis, Fasciola hepatica, Schistosoma* spp. were also identified in the soil samples. All soil samples had the following bacteria genera present in them: *Escherichia, Bacillus, Staphyloccus, Pseudomonas* and *Clostridium*. The upper soil profile had higher counts of coliforms and

helminth eggs than the deeper soil profile. Wastewater irrigated plots had higher numbers of coliforms and helminth counts than those obtained from the potable water irrigated and no irrigation plots. The study concluded that pathogenic microorganisms in soils irrigated with wastewater may extend into lower profiles of the soil. However, factors such as the sieving effect of soil (influenced by soil texture), higher pathogen die-off rates, lack of substrate diversity and reduced soil moisture contributed to lower counts of coliforms and helminthes in the lower profiles of soil.

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LIST OF ABBREVIATIONS

CDC	Center for Disease Control
CFU	Colony Forming Unit
FAO	Food and Agricultural Organization
FC	Fecal Coliforms
GSS	Ghana Statistical Service
IMWI	International Water Management Institute
KNUST	Kwame Nkrumah University of Science and Technology
MPN	Most Probable Number
UPA	Urban and Peri-urban Agriculture
US-EPA	United States – Environmental Protection Agency
WHO	World Health Organization
PW	Potable water
TC	Total Coliforms

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CHAPTER ONE

1.0 INTRODUCTION

More than 10% of the world's population consume foods produced by irrigation with wastewater (WHO, 2006). The percentage will be considerably higher among populations in low-income countries with arid and semi-arid climates. Both treated and untreated wastewaters are used directly and indirectly for irrigation in developed and less developed countries (WHO, 2006). Overall, population growth will be the main driving force for a further demand on water resources, and increased wastewater use.

Wastewater re-use may be 'direct' or 'indirect'. In most developing countries direct wastewater use projects are normally centred near large metropolitan areas. These schemes often only use a small percentage of the wastewater generated. The result is that indirect use of wastewater prevails in most developing countries (Wescott, 1997).

Indirect use occurs when treated, partially treated or untreated wastewater is discharged to reservoirs, rivers and canals that supply irrigation water for agriculture. Indirect use poses the same health risks as planned wastewater use projects, but may have a greater potential for health problems because the water user is unaware of the wastewater being present. Indirect use is likely to expand rapidly in the future as urban population growth outstrips the financial resources to build adequate treatment works. Where indirect use occurs, the primary objective must also be to ensure that it is in a manner that minimizes or eliminates potential health risks (Wescott, 1997).

In Ghana, urban sanitation infrastructure is poor and only a small portion of the (primarily domestic) wastewater is collected for treatment. The bulk ends up in drains and nearby water bodies and is used by urban and peri-urban vegetable farmers for irrigation. From a general survey among open-space farmers carried out in 2002, it was found that about 84% of nearly 800 farmers farming in and close to Accra and almost all 700 farmers in the city of Tamale used wastewater for irrigation, at least during the dry seasons (Keraita and Drechsel, 2004).

Wastewater irrigated vegetable production is the dominant agricultural practice in Kumasi. An instance is the agricultural practice in Atonsu**.** This practice of untreated wastewater irrigation has been going on for over 20 years. The primary crops grown are lettuce, tomatoes, onions and cabbage. Vegetables are preferred because they grow faster and are more economically profitable.

In Kumasi, wastewater flows from drains into streams, which are usually used for irrigation. Thus wastewater is mostly used in a diluted form mixed with surface runoff and/or stream water (Cornish *et al.,* 2001). Nonetheless, there are instances where farmers use wastewater directly from drains and broken sewers without further dilution, especially in the dry season. For omission of superfluous details and reduced complexity, the term 'wastewater' will be adopted to refer to all these sources of wastewater (Keraita and Drechsel, 2004).

Open-space urban and peri-urban vegetable farming in Kumasi is market-oriented and depends on water availability. It not only supports the livelihoods of many farmers and traders but also contributes significantly to the supply of perishable vegetables to cities. Wherever space allows, urban and peri-urban agriculture takes advantage of any water source, be it polluted or not, for

dry-season or annual irrigated farming. As most of the wastewater is of domestic origin, faecal coliforms are the contaminants of primary concern.

Several studies have reported increase in the yield of crops irrigated with wastewater (Ouazzani *et al.,* 1996; Jamjoum and Khattari, 1986). Soilborne plant pathogens can significantly reduce yield and quality in vegetable crops. Measurement of these pathogens are particularly challenging because they often survive in soil for many years and each vegetable crop may be susceptible to several species of pathogens. Most of the pathogens in soil and wastewater are enteric in origin. They include viruses, bacteria, protozoan parasites and helminths.

1.1 PROBLEM STATEMENT

The health hazards associated with direct and indirect wastewater use are of two kinds: (i) the rural health and safety problem for those working on the land or living on or near the land where the wastewater is being used, and (ii) the risk that contaminated products from the wastewater use area may subsequently infect humans or animals through consumption or handling of the foodstuff or through secondary human contamination by consuming foodstuffs from animals that used the area (WHO, 2006; Wescott, 1997).

Though heavy metal levels in water bodies in and around Kumasi's urban centres are not high (McGregor *et al.,* 2001; Mensah *et al.,* 2001; Cornish *et al.,* 1999), studies also showed that nutrients and microbiological contaminants in irrigation water sources in most cases exceeded the WHO guidelines significantly (Keraita *et al.,* 2003). Keraita and Drechsel (2004) reported that faecal coliforms typically reached values of 10^6–10^8 cells per 100 ml while total coliform

levels often range from 10^8–10^{10} cells per 100 ml in Kumasi. Market surveys in Kumasi showed that it is very difficult to find any irrigated vegetables (e.g. lettuce, spring onions, cabbage) that are not contaminated with faecal coliforms. Helminth eggs are also commonly found on such vegetables (Keraita *et al.*, 2003).

Microbial pathogens that can be potentially found in soil and wastewater are enteric (transmitted by fecal-oral route) in origin and include bacteria, viruses, protozoa and viruses. Gastrointestinal infections, for example, are the most common diseases caused by enteric bacteria (Toze, 1997). Some examples include salmonellosis (*Salmonella sp.*), cholera (*Vibrio cholera*), dysentery (*Shigella sp.*) and other infections caused by *Campylobacter jejuni*, and *Escherichia coli* O157:H7 and many other strains.

Humans are in contact with soil permanently, either directly or indirectly via food, water and air; and thus soil may act as a vector and source of important human disease agents (Santamaria and Toranzos, 2003). Diseases associated with soil have been classified depending on the origin (Santamaria and Toranzos, 2003). These include: (i) soil-associated diseases which are caused by opportunistic or emerging pathogens that belong to the normal soil microbiota (e.g. *Aspergillus fumigates* is a very common fungus occurring in soils and can infect the lungs via inhalation of spores), (ii) soil-related diseases, which result in intoxication from the ingestion of food contaminated with entero- or neurotoxins (*Clostridium botulinum, C. perfringens* and *Bacillus cereus* are some of these pathogens), (iii) soil-based diseases caused by pathogens indigenous to soil (which include *C. tetani, B. antharacis*, and *C. perfringens*) and (iv) soil-borne diseases caused by enteric pathogens which get into soil by means of human or animal excreta. Enteric pathogens transmitted by the fecal-oral route are bacteria, viruses, protozoa and helminths.

Although a lot of studies have been done on pathogens and diseases associated with wastewater-irrigated vegetable production in Kumasi (Keraita *et al.,* 2003; Keraita and Drechsel, 2004; Amoah *et al.,* 2005), enteric diseases and their link to soil have been understudied and possibly underestimated. This study therefore sought to increase knowledge in this area by determining the effects of wastewater irrigation on targeted soil microbial populations.

1.2 JUSTIFICATION

As part of expanding the protection of the health of the population, it is important to assess the sanitary quality of soil and water used for irrigated vegetable production and other purposes, using laboratory analyses in order to obtain information such as the concentration of certain pathogenic micro-organisms or, to establish their presence or absence (Razzolini and Nardocci, 2006).

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

Understanding the microbiology of the soils used in vegetable production is therefore necessary to establish the potential risks that farm workers and consumers of these food products are exposed to. Without studies on the ecology of enteric pathogens in soils, a true characterization

of public health risk as a result of direct or indirect exposure to soils will be impossible (Santamaria and Toranzos, 2003).

1.3 OBJECTIVES

The objective of this study was to determine the effect of irrigation with wastewater on the microbiology of soils under vegetable cultivation.

The specific objective was to assess the soil microbial contamination levels at different soil profiles in wastewater irrigated agricultural plots in urban Kumasi.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wastewater Reuse in Irrigated Agriculture

The use of urban wastewater in agriculture is a centuries-old practice that has received renewed attention due to increasing scarcity of freshwater resources in many arid and semi arid regions. Driven by rapid urbanisation, growing wastewater volumes and cost of water treatment, wastewater is widely used as a low-cost alternative to conventional irrigation water. It supports livelihoods through the generation of considerable income that is derived from urban and peri-urban agriculture. These attributes tend to overshadow the health and environmental risks associated with wastewater use. Though pervasive, this practice is largely unregulated in low-income countries, and the costs and benefits are poorly understood (Scott *et al.*, 2004).

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. The report also showed estimates of median levels of treated wastewater in Asia to be about 35% and 14% in Latin America and Caribbean, respectively but an abysmal 0% in sub-Saharan Africa (SSA). Hence, large amounts of untreated wastewater being discharged into urban drainage systems and other natural waterways are used by farmers in these countries.

Direct and indirect wastewater use in agriculture occurs in most developing countries. Direct wastewater irrigation practices are normally centered near large metropolitan areas (Wescott, 1997), but only a small percentage of the wastewater generated is used directly. Rather, indirect

use of wastewater prevails in most developing countries (Wescott, 1997). Indirect use occurs when treated, partially treated or untreated wastewater contaminate surface water that supply irrigation water to agriculture. Indirect wastewater reuse poses health and environmental problems of the same nature and magnitude as those associated with direct wastewater use in agriculture (Wescott, 1997). There is a growing recognition that the volumes of wastewater produced will increase as an outcome of continued urbanization. Therefore wastewater use needs to be better incorporated into the overall management of water resources (WHO, 2006).

2.2 Opportunities associated with wastewater irrigation

2.2.1 The resource potential of wastewater

Wastewater is not only a fertiliser. Its organic matter content, which serves as a soil conditioner and humus replenisher, – an asset not shared by chemical fertilisers – is of agricultural importance. Urban farmers in arid or semi-arid zones or during dry seasons, in addition to procuring water for irrigation, are endeavouring to get access to wastewater, raw or treated. This allows them to renounce or minimize the purchase of chemical fertiliser (Strauss, 2000).

Opportunities exist as sewage effluents from municipal origin are rich in organic matter and also contain appreciable amounts of major and micronutrients (Feign *et al*., 1991). Accordingly nutrient levels of soils are expected to improve considerable with continuous irrigation with sewage (Baddesha *et al.*, 1986).

2.2.2 Socioeconomic opportunities

Wastewater reuse for agriculture is also necessary to meet growing water demands and conserve current potable supplies in most municipalities in Ghana. All over the world the practice of wastewater irrigation is an important part of water resource planning (Wang, 1983; Asano 1998; Rattan *et al.*, 2005; Kalavrouziotis and Drakatos, 2001). Wastewater can be utilized for the irrigation of a variety of field crops and gardens in regions with limited natural water for agricultural purposes (Oron *et al.*, 1992).

The practice of wastewater reuse can also be seen as a suitable disposal of waste products and a means of providing a reliable supply of irrigation water. The practice thus increases urban supply of foods, particularly vegetables and some staple crops.

2.3 Quality of Irrigation Water Used in Wastewater Irrigated Farming

It is very important to be aware of the health hazards that may result from the reuse of wastewater in irrigated farming, despite the potential benefits. Wastewater is a carrier of bacteria, viruses, protozoa and nematodes, which can cause various diseases, a situation met especially in some developing countries, where they use partially processed wastewater, for crop irrigation (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; Melloul *et al.*, 2001; Al-Lahham *et al.*, 2003; Amahmid *et al.*, 1999).

2.3.1 Microbial contaminants in wastewater

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.*, 1986a).

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). Examples of the different microbial pathogens are given in Table 2.1. 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).

2.3.1.1 Viruses

Viruses are the smallest of the pathogens found in water. One unit is basically a tiny bundle of genetic material—either DNA or RNA—carried in a shell called the viral coat, or capsid, which

is made up of bits of protein called capsomeres. Some viruses have an additional layer around this coat called an envelope (ASM, 2006). Viruses can't metabolize nutrients, produce and excrete wastes, move around on their own, or even reproduce unless they are inside another organism's cells. They are not cells on their own and are present in water as inactive particles. Yet viruses have played key roles in shaping the history of life on our planet by causing diseases in animals and plants. They have become culprits in many human diseases including common cold, flu, gastroenteritis and Hepatitis A which has become increasingly linked with viral infections resulting from consumption of contaminated vegetables.

Raw or untreated wastewaters contain a range of pathogenic viruses which poses threat to human health. Viral numbers detected in wastewaters are in excess of 10^2-10^6 particles. Viruses require lower infection doses to than most of the other pathogenic microbes (Yates and Gerba, 1998). Viruses are also generally more difficult to detect in environmental samples such as wastewater (Toze, 1997).

Enteric viruses are groups of viruses which are usually found in the intestinal tracts of humans and viruses. They are mainly associated with diarrhoea and gastroenteritis. The majority of these viruses can be commonly detected in faecal contaminated water. Waterborne enteric viruses threaten both human and animal health. These pathogens are host specific and cause a wide range of diseases and symptoms in humans or other animals (Cruz *et al*., 1996, Theng and Lipp, 2005, Haas *et al.*, 1999).

Astroviruses Gastroenteritis, for instance, is caused by many viruses including rotaviruses, adenoviruses and astroviruses (Table 2.1).

2.3.1.2 Protozoan parasites

A protozoan parasite is basically a protozoan that has adapted to invade and live in cells and tissues of other organisms. They are mostly present in food, soil, and water.

In general protozoan parasite cysts (the resulting stage of the organism which is found in sewage) are larger than bacteria, although they can range in size from 2 μm to over 60 μm. Protozoan parasites are present in faeces of infected persons; however they can also be excreted by healthy carriers (Yates, 1993). Protozoa parasites commonly detected in wastewater and wastewater irrigated fields are the *Giardia*, *Enterobius vermularis*, *Entamoeba hystolytica*, *Cryptosporium parvum* (Mintz et al. 1993, Erdogrul and Sener 2005).

The cysts of *Cryptosporidium* are of increasing importance because of their presence in water supplies. Passage through the stomach, or in this case chlorine bleach, weakens the wall of a cyst (left, circled). When in the gut, four spindle-shaped motile sporozooites burst from the cyst to infect gut epithelial cells and continue their life cycle.

Entamoeba histolytica, another water-borne pathogen, can cause diarrhea or a more serious invasive liver abscess. When in contact with human cells, these amebae are cytotoxic. There is a rapid influx of calcium into the contacted cell, it quickly stops all membrane movement save for some surface blebbing (Sullivan, 2006). Internal organization is disrupted, organelles lyse, and the cell dies. The amoeba may eat the dead cell or just absorb nutrients released from the cell.

Giardiasis can also be contracted via contaminated foods. *Giardia* uses a ventral suction cup, seen in the differential interference contrast image (right), to attach to its host's intestinal

epithelium. There is some evidence that a heavy infection of attached *Giardia* physically blocks the important transport of nutrients across the epithelium (Sullivan, 2006).

Most protozoan cysts are excreted in irrigation water sources. This poses and increases risk of disease to wastewater irrigated farmers and consumers when viable organisms in soils or irrigation water come into contact with the farmers and minimally processed crops.

2.3.1.3 Helminths

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). Table 2.1 shows some of the commonly detected helminth parasites in soils irrigated with wastewater. These 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 particulary 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).

Table 2.1: Examples of Microbial Pathogen levels and diseases associated with untreated wastewater

Pathogen by Taxon	Disease	Concentrations in wastewater	Infectious dose ¶
Viruses			
Enteroviruses			
Poliovirus	Poliomyelitis		
Enterovirus	Gastroenteritis, heart		
Echovirus	Anomalies,meningitis		
Hepatitis A virus	Hepatitis		
Adenovirus	Respiratory disease	10^2-10^6	Low
Calicivirus			
Norwalk agent	Gastroenteritis		
SSRV	Diarrhoea, vomiting, fever		
Rotavirus	Gastroenteritis		
Astrovirus	Gastroenteritis		
Bacteria			
Shigella spp.	Shigellosis		Low
Salmonella typhi	Typhoid,		High
Vibro cholera	Salmonellosis		High
Escherichia coli	Cholera	10^0-10^{10}	High
Yersinia enterocolitica	Gastroenteritis		High
Shigella dysinterae	Yeriniosis		Low
Campylobacter jejuni	Dysentery		High
	Gastroenteritis		
Protozoans			
Cryptosporidium	Diarrhoea, fever		
parvum	Giardiasis	10^0-10^5	Low
Giardia intestinalis	Amoebiasis (amoebic		
Entamoeba histolytica	dysentery)		

¶ Low indicates only a few viral particles/cells/cysts/eggs required to cause infection.
High indicates many required to cause an infection.
Source: Adopted from Toze (1997) and Ottoson (2005)

Continuation of Table 2.1

Pathogen by Taxon	Disease	Concentrations in wastewater	Infectious dose ¶
Helminths			
Ascaris lumbricoides (Round worm)	Ascarisis	10^0-10^5	Low
Enterobius vericularis (Pin worm)	Enterobiasis		
Taenia saginata (Tapeworm)	Taeniasis		
Trichuris trichiura (Whip worm)	Trichuriasis		
Strongyloides stercoralis	Strongyloidasis		

¶ Low indicates only a few viral particles/cells/cysts/eggs required to cause infection.
High indicates many required to cause an infection.
Source: Adopted from Toze (1997) and Ottoson (2005)

2.3.1.4 Bacteria

Bacteria are ubiquitous in nature. They are found in water, soil, organic matter, and living bodies of plants and animals (Gerardi and Zimmerman, 2005). They are the most common of the microbial pathogens found in wastewaters and soils (Toze, 1997). There is a broad range of bacterial pathogens and opportunistic pathogens, which can be detected in wastewaters.

Most bacteria are harmless, and many of them are enteric in origin, i.e. they colonize the digestive tract of humans and animals. However, bacterial pathogens which cause non-enteric illnesses (e.g., *Legionella* spp., *Mycobacterium* spp., and *Leptospira* spp.) have also been detected in wastewaters (Neumann *et al*., 1997; Wilson and Fujioka, 1995; Fliermans, 1996; Wei *et al*., 1995; Okafo *et al*., 2003). Enteric bacteria include *Shigella* spp., *Salmonella typhii*, *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus*

cereus, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Campylobacter jejuni* and *Clostridium botulinum*.

Gastrointestinal infections are the most common diseases caused by enteric bacteria. Some examples are Salmonellosis (*Salmonella spp.*), cholera (*Vibrio cholera*), and other infections caused by *Campylobacter jejuni*, *Yersinia spp.* and *Escherichia coli* O157:H7 and many other strains (Santamaria and Toranzos, 2003). Dysentery is caused by a number of various *Shigella* species, some *Salmonella* species as well as some strains of enteropathogenic *E. coli* (Grant *et al.*, 1996). Typhoid, a disease caused by *Salmonella typhi* and other closely related *Salmonella* species, has been traced in food stuffs irrigated with wastewater (Bryan, 1997).

Non-enteric bacterial diseases which can be transmitted by pathogens present in wastewater include legionellosis (Legionnaire's disease) a potentially fatal pneumonia caused by *Legionella* species; leptospirosis, a zoonotic infection causing a febrile illness caused by *Leptospira interrogans*; and melioidosis, a pneumonia-like disease caused by by *Pseudomonas pseudomallei* (Toze, 1997).

Opportunistic pathogens (microorganisms which cause infections and disease under optimal conditions, commonly in the very young, elderly and immunocompromised) commonly found in wastewaters include *Pseudomonas*, *Streptococcus*, *Flavobacterium* and *Aeromonas* species (Ashbolt *et al.*, 1995). They are often members of the natural populations and have the ability to rapidly increase in number when given sufficient nutrients (Toze, 1997). Wastewaters and soils irrigated with wastewater often have high nutrient loads, thus high numbers of these opportunistic pathogens can be present, increasing the risk of infections occurring from them.

Clostridium perfringes, *Staphylococcus aureus* and some other bacteria have been linked with food poisoning. They cause intestinal disorder characterized by sudden onset of abdominal colic followed by diarrhea (Gerardi and Zimmerman, 2005).

2.4 Microbial indicators in soil and wastewater

The use of indicator microorganisms in determining the relative risk of the presence of pathogenic microorganisms in a sample has become necessary to avoid the difficult, time consuming and hugely expensive undertakings that will be needed for the detection, isolation and identification of the many different types of microbial pathogens known to contaminate soil and groundwater (Toze, 1997). Cabelli (1977) noted that the best indicator organism should be the one whose densities correlate best with health hazards associated with one or several given types of pollution sources. He also listed the requirements for an indicator as follows: (a) the indicator should be consistently and exclusively associated with the source of the pathogens, (b) it must be be present in sufficient numbers to provide an accurate density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable, (c) it should approach the resistance to disinfectants and environmental stress, including toxic materials deposited therein, of the most resistant pathogen potentially present at significant levels in the sources, and (d) it should be quantifiable in recreational water by reasonably facile and inexpensive methods and with considerable accuracy, precision, and specificity.

Historically, the faecal coliforms, in particular *E. coli*, have been used as indicators of faecal contamination of water sources (APHA, 1989). Fecal coliform bacteria are a sub-group of total coliform bacteria. They appear in great quantities in the intestines and feces of people and

animals. The presence of fecal coliform in a drinking water sample often indicates recent fecal contamination – meaning that there is a greater risk that pathogens are present than if only total coliform bacteria is detected. Faecal coliforms which have been excreted by warm blooded animals can be grown on selective media at 44.5^{o}C Toze (1997). This ability to be cultured at higher temperatures has earned them the name thermotolerant coliforms (TTC) and also made them mainstay indicators in the water industry.

E. coli is a sub-group of the fecal coliform group. Most *E. coli* bacteria are harmless and are found in great quantities in the intestines of people and warm-blooded animals. Some strains, however, can cause illness. The presence of *E. coli* in a drinking water sample almost always indicates recent fecal contamination – meaning there is a greater risk that pathogens are present. *E. coli* outbreaks receive much media coverage. Most outbreaks have been caused by a specific strain of *E. coli* bacteria known as *E. coli O157:H7*. When a drinking water sample is reported as "*E. coli* present" it does not mean that this dangerous strain is present and in fact, it is probably not present. However, it does indicate recent fecal contamination. Boiling or treating contaminated drinking water with a disinfectant destroys all forms of *E. coli*, including *O157:H7.*

Other potential bacterial indicators of presence of microbial pathogens include enterococci, bifidobacteria, and Bacteroides (Baker & Bovard, 1996). The enterococci have been considered to possibly be useful as secondary indicators of faecal contamination of water sources (APHA, 1989; Lecierc *et al.*, 1996). The enterococci are generally a little more resistant than the faecal coliforms to treatment processes and environmental factors (Toze, 1997). Studies comparing

different potential indicators, however, have indicated that enterococci are not as accurate as other potential indicators (Ferguson *et al.*, 1996; Jagals *et al.*, 1995).

2.5 Environmental and health risks associated with wastewater reuse

The practice of wastewater irrigated agriculture threatens public health and the environment, and possibly limits its long-term sustainability. Risks associated with this type of farming involve the risk exposed to all individuals involved with the vegetable production, marketing and consumption.

2.5.1 Health Risks

While recycling and reuse of wastewater for agriculture, industry and non-potable urban purposes can be a highly effective strategy for developing a sustainable water resource in water short 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.*, 1986b). 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 reuse 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). Faruqui

et al. (2004) also reported that in Pakistan, farmers using raw wastewater are five times more likely than those using canal water to be infected by hookworms. Living in the small intestine, hookworms cause heavy blood losses, and anaemia and retardation in children (Ensink *et al.*, 2004). 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).

Bacterial and viral infections are other health threats which can occur after the consumption of raw vegetables contaminated with faecal matter. Cholera epidemic in Jerusalem, and typhoid epidemics in Santiago and Dakar are all isolated to urban and peri-urban agriculture (UPA) (Faruqui *et al*., 2004).

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).

2.5.2 Environmental Impacts

Sewage effluents from municipal origin are rich in organic matter and also contain appreciable amounts of major and micronutrients (Pescod, 1992; Brar *et al.*, 2000). However, these chemical constituents may affect public health and/or environmental integrity (Assadian *et al.,* 2005).

Micronutrient concentration levels may be very high in the municipal wastewater, contributing to surface water eutrophication and accumulation of organic matter in the soil (Rattan *et al.*, 2005).

The wastewater may also contain significant quantities of toxic metals (Som *et al.*, 1994; Yadav *et al.*, 2002) and therefore its long-term use may result in toxic accumulation of heavy metals with unfavorable effects on plant growth (Rattan *et al.*, 2005). Farming products produced in regions of high micronutrient and heavy metal content may have adverse effects on human health due to the high level of these metals in the edible plant part (Tiller, 1986; Mapanda *et al.*, 2005).

There are cases in China where irrigation with industrial wastewater has been associated with a 36% increase in enlarged livers and 100% increases in both cancer and congenital malformation rates, compared to control areas where industrial water was not used for irrigation (Yuan, 1993). In Japan, chronic cadmium poisoning as a result of wastewater use has caused Itai-itai disease, a bone and kidney disorder (WHO, 1992).

2.5.3 Soil contamination

Humans are in contact with soil permanently, either directly or indirectly via food, water and air; and thus soil may act as a vector and source of important human disease agents (Santamaria and Toranzos, 2003). Although many of the diseases associated with soils have been well characterized and studied, enteric diseases and their link to soil contamination have been understudied and possibly underestimated (Santamaria and Toranzos, 2003).

The practice of wastewater reuse could result in soil damage. Although the organic matter in wastewater can help improve soil texture and water-holding capacity, wastewater also has

harmful effects, particularly in arid environments, by causing soil salinisation, blocking soil interstices with oil and grease, and accumulating heavy metals (Faruqui *et al.*, 2004). One example is in the case of Pakistan where over-applied wastewater with insufficient drainage has resulted in signs of degrading soil structure, visible soil salinity, and the delayed emergence of wheat and sorghum due to an excess of nutrients indirectly applied (Faruqui *et al.*, 2004).

2.5.4 Outbreaks of soil-borne diseases

It is estimated that more than 1 billion people in the world are infected by soil-transmitted helminths (STH) (*Ascaris lumbricoides, Trichuris trichiura* and hookworm) (Crompton 1999). For example, in the United States, waterborne diseases were caused by contaminated ground water (Craun and Calderon, 1996; Morbidity and Mortality, 2000). A cryptosporidiosis outbreak in the United Kingdom with 47 reported cases had a strong statistical correlation with two groundwater sources (Santamaria and Toranzos, 2003). Fruits and vegetables frequently come in contact with soil post-harvest and thus may become contaminated with soil enteric pathogens present in sewage sludge or manure spread (Santamaria and Toranzos, 2003). Shuval *et al.* (1984) showed that an outbreak which occurred as a result of vegetables irrigated with wastewater was linked with *Vibrio cholera* present in the irrigated soils.

Ingestion of soil, or geophagia, is another way in which humans, and especially infants and young children, can become infected with enteric pathogens (Toranzos and Marcus, 1997). Although geophagia is the voluntary ingestion of soil, involuntary ingestion as a result of wind could present a risk to immunocompromized individuals and other special populations (Lagoy, 1987; Toranzos and Marcus, 1997).

Table 2.2 Summary of health risks associated with the use of wastewater for irrigation

Group exposed	Health threats		
	Helminths	**Bacteria/viruses**	**Protozoa**
Consumers	Significant risks of helminth 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 diarrhea when water quality exceeds 10^4 thermotolerant coliforms per 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 risks of helminth infection for both adults and children in contact with untreated wastewater; increased risk of hookworm infection to workers who do	Increased risk of diarrhoeal disease in young children with wastewater contact if water quality exceeds 10^4 thermotolerant coliforms per 100 ml; elevated risk of *Salmonella*	Risk of *Giardia intestinalis* infection reported to be insignificant for contact with both untreated and treated wastewater; another study in Pakistan estimated a

Source: WHO (2006)

Continuation of Table 2.2

Group exposed	Health threats		
	Helminths	**Bacteria/viruses**	**Protozoa**
Nearby communities	Transmission of helminth infections 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 total coliforms/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 is not associated with increased viral infection rates	No data for transmission of protozoan infections during sprinkler irrigation with wastewater

Source: WHO (2006)

2.6 Pathogen Dispersion and Movement in Surface and Subsurface Soils

The movement and survival of microorganisms in soil and the subsurface is a highly complex issue which depends on the pathogen type, soil type and conditions, water characteristics, temperature, light availability, the composition and viability of the indigenous microbial population, and the geographical conditions (e.g. Tropical, temperate, or desert) (see Table 2.3).

Microbial movement in soils is also dependent on the water saturation state (Santamaria and Toranzos, 2003). Microorganisms move rapidly under saturated conditions, but only for a few centimeters, because microorganisms are in close contact with soil particles, promoting the adsorption of microorganisms onto the soil particles (Santamaria and Toranzos, 2003). All pores in soil are filled with water when soil is saturated, allowing microorganisms to pass through the soil.

One of the major influences of soils is as filters, which is dependent on pore sizes and grain size (Toze, 1997). Thus, soil texture controls, in part, the movement of microorganisms, because finegrained soils avoid movement while coarse-grained soils promote it (Sinton, 1986; Abu-Ashour *et al.*, 1994).

Adsorbable material also influences the movement of microorganisms in soil (Toze, 1997). Toze (1997) argued that the degree of adsorption is dependent on the soil composition (i.e. clay content, % of iron hydroxides present etc), the presence of organic matter, cation concentration, and pH. Organic matter present in the soil matrix tends to compete with bacterial cells and viral particles for adsorption sites and thus increases the transport of microorganisms through the soil matrix (Johnson and Logan, 1996; Powelson *et al*., 1991).

Soil composition and pH influence the adsorptive ability of the soil matrix (Toze, 1997). Experiments have shown that fine-grained colloidal material was ten times more effective in adsorbing viruses than sand particles (Matthess *et al.*, 1988). There is increased sorption at acidic or neutral pH and little adsorption at pH values above 8 (Toze, 1997).

Another important environmental factor affecting microbial movement is rainfall. It can result in pathogen spread by runoff from places where manure or biosolids have been applied or by leaching through the soil profile (Santamaria and Toranzos, 2003). Movement of bacteria and viruses through the soil and the subsurface has been observed to rapidly increase during heavy rainfall (Gerba and Bitton, 1984). In Quebec, Canada, human and pig enteroviruses were isolated from 70% of the samples collected from a river. The contamination source was attributed to a massive pigraising activity in the area (Payment, 1989).

Table 2.3 Factors affecting movement of viral particles and bacteria in soil and groundwater

Factor	Virus	Bacteria
Soil type	Pore size has an influence. Iron oxides increase the adsorptive capacity of soils. Muck soils are generally poor adsorbents. The presence of clays can retard movement	Pore size is important for filtration of bacterial cells. Clay particles retard movement
pH	Adsorption increases as pH decreases	Adsorption increases as pH decreases
Cations	Adsorption increases as cation concentration increases	Adsorption increases as cation concentration increases

Source: Toze (1997) as summarized from Gerba and Bitton (1984), Yates and Yates (1988), and Roper and Marshall (1979).

Continuation of Table 2.3

Factor	Virus	Bacteria
Soluble organics	Increasing concentration of organic matter decreases viral adsorption	Incrases in organic matter can retard bacterial cell movement. Organic matter may also compete for adsorption sites
Flow rate	Increased flow rates decrease viral adsorption	Increased flow rates decrease bacterial adsorption
Saturated vs unsaturated flow	Viral movement decrease under unsaturated flow conditions through increased adsorption	Bacterial movement decrease under unsaturated flow conditions due to loss of water in larger pore spaces
Microbial factors	Adsorption to soils varies with viral species. Different viruses may have different isoelectric points	Motile bacterial cells move faster than non-motile cells. The possession of appendages can increase adsorption capacity. Size and shape of the bacterial cell.

Source: Toze (1997) as summarized from Gerba and Bitton (1984), Yates and Yates (1988), and Roper and Marshall (1979).

Table 2.4 Survival times of selected excreted pathogens in soil, wastewater and on crop surfaces at 20–30°C

Type of pathogen	Survival Time (in days unless otherwise stated)		
	In soils	**On crops**	**In wastewater**
Viruses			
Enteroviruses[a]	<100 but usually<20	<60 but usually <15	<120 but usually<50
Bacteria			
Faecal coliform	<70 but usually <20	<30 but usually <15	<60 but usually <30
Salmonella spp	<70 but usually <20	<30 but usually <15	<30 but usually <10
Vibrio cholera	<20 but usually <10	<5 but usually <2	<30 but usually <10
Protozoa			
Entamoeba histol.	<20 but usually <10	<10 but usually <2	<30 but usually <15
Helminths			
Ascaris lumbricoides eggs.	Many months	<60 but usually <30	Many months
Hookworm larvae	<90 but usually <30	<30 but usually <10	
Taenia saginata eggs	Many months	<60 but usually <30	
Trichuris trichiura eggs	Many months	<60 but usually <30	

[a] Includes polio-, echo-, and coxsackieviruses.
Source: Faechem *et al.* (1983)

2.7 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. Survival can be related to the potential microbial types present, wastewater applications, health risk analysis etc. Pathogenic microorganisms remain a health risk as long as they persist in environments such as wastewater. The longer they survive in an environment the greater the potential they have of becoming mobilized if the chemical, physical or hydraulic conditions are suitable. Increased persistence and survival also increases the chance of their dispersion due to application procedures, for example spray irrigation. Therefore, the longer pathogens persist in wastewater, the chance that they could come into contact with workers and the general public increases". The literature on survival times of excreted pathogens in soil and on crop surfaces has been reviewed by other authors (Faechem *et al.*, 1983; Strauss, 1985) as well.

The survival of pathogens is affected by the type of organism, the presence of other antagonistic organisms, the soil characteristics, temperature, moisture, nutrients, pH, and sunlight (see Table 2.5). Wide variability in reported survival times reflects the influence of environmental and analytical factors (Wescott, 1997).

Some organisms are more resistant than others (Salvato *et al.,* 2003). Soil moisture favors the survival of viruses and bacteria (Santamaria and Toranzos, 2003). Soil moisture of about 10 to 20 percent of saturation appears to be best for survival (Salvato *et al.,* 2003). Reductions in bacterial and viral population densities 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 (Gerba and Bitton, 1984; Toze, 1997; Salvato *et al.*, 2003).

Viral survival may be longer than bacterial survival and is greatly increased at lower temperature (Wescott, 1997). Generally, lower temperatures favor pathogen survival (Toze, 1997; Santamaria and Toranzos, 2003, Salvato *et al.*, 2003). In laboratory studies, as the temperature was increased from 15^{o}C to 40^{o}C, the inactivation rate increased significantly for poliovirus type 1 (Straub *et al.*, 1992).

The sorption 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 bacterial cells, and possibly viral particles, by creating a barrier against microbial predators and parasites (Santamaria and Toranzos, 2003; Roper and Marshall, 1978). Hence, survival rates of enteric pathogen are lower in sandy soils with a low water-holding capacity.

Santamaria and Toranzos (2003) in their report stated that pH affects the adsorption characteristics of cells, so inactivation rates in acidic soils are lower. They argued that increases in cation concentrations also result in increased adsorption rates, consequently affecting microbial survival. They also mentioned that soluble organics increase survival and, in the case of bacteria, may favor their regrowth 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); they 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 (Table 2.4).

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 crop growing cycles as is often the case with vegetables (Table 2.4). While the length of the crop growing cycle is important, equally important is the length of time since the last irrigation cycle (potential exposure cycle) (Wescott, 1997). The excreted pathogens, if they do enter an irrigated area with the irrigation water, have the potential to remain infectious for a considerable period of time thus steps must be taken to interrupt this infection cycle WHO (1989).

Table 2.5 Factors affecting survival time of pathogens in soil

Factor	Comment	Effect on bacterial survival
Antagonism from soil microflora	Antagonistic effects from bacteria or algae may enhance die-off; bacteria may be preyed upon by protozoa	Increased survival time in sterile soil
pH	Some viruses survive longer in lower pH soils, while alkaline soils are associated with more rapid die-off of viruses; neutral to slightly alkaline soils favour bacterial survival	Shorter survival time in acid soils (pH 3-5) than in alkaline soils
Temperature	Most important factor in pathogen die-off. High temperatures lead to rapid die-off; low temperatures lead to prolonged survival. Freezing temperatures can also cause pathogen die-off.	Longer survival at low temperatures; longer survival in winter than in summe
Sunlight (UV radiation)	Direct sunlight leads to rapid pathogen inactivation through desiccation and exposure to UV radiation	Shorter survival time at soil surface

Source: Shuval *et al.* (1986a) as adapted from Gerba *et al.* (1975), Strauss (1985)

Continuation from Table 2.5

Factor	Comment	Effect on bacterial survival
Soil Content (Organic matter)	Clay soils and soils with high organic content favour survival	Increased survival and possible regrowth when sufficient amounts of organic matter are present
Foliage/plant type	Certain plants have sticky surfaces (e.g., zucchini) or can absorb pathogens from the environment (e.g., lettuce, sprouts) leading to prolonged survival of some pathogens; root crops such as onions are more prone to contamination and facilitate pathogen survival	Increased absorption from the environment facilitates prolonged survival of some pathogens
Moisture-holding capacity	Humid environments favor pathogen survival ; Dry environments facilitate pathogen die-off	Survival time is less in sandy soils than in soils with greater water-holding capacity
Moisture content	Humid environments favor pathogen survival ; Dry environments facilitate pathogen die-off	Greater survival time in moist soils and during times of high rainfall

Source: Shuval *et al.* (1986a) as adapted from Gerba *et al.* (1975), Strauss (1985)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Research design

The research was designed to assess the levels of microbial contamination in soils in Atonsu, an urban vegetable production site in Kumasi. Microbial quality of samples from different irrigation water sources as well as the soils used for the vegetable production were assessed and analysed. The soils analysed were sampled from fields treated with different irrigation water sources. The treatments include plots where:

(i) wastewater irrigation practice has been going on for over a decade

(ii) piped-water (PW) irrigation practice has been used for over a decade

(iii) no recent cultivation has taken place (non-irrigated plots)

PW-irrigated plots and non-irrigated plots were treated as controls.

Plots used for producing lettuce were chosen because the cultivation requires a lot of water from the soil. Another reason is the rather high intensification of wastewater irrigated lettuce farming.

3.2 Study site and description

The experiments were conducted on urban vegetable farming sites at Atonsu, Kumasi. Kumasi is the capital town of the Ashanti Region and the second largest city in Ghana, with a population over one million (Amoah *et al.*, 2005; GSS, 2002). The site supplies vegetable products to the inhabitants of Kumasi and its environs. The sources of irrigation water on the site include potable

(pipe-borne) water and wastewater from shallow (approximately 1 meter deep) hand dug wells and stream. The stream serves as a source of irrigation water for the farmers as well as water for domestic purposes for nearby populations. The types of crops grown on the site include lettuce, spring onions and cabbage.

3.3 Sampling

3.3.1 *Soil sampling for microbiological analysis*

Soil samples were taken from the top 0 – 30 cm layer. Most microorganisms are found in this zone, and the conditions here are usually different from the profiles beneath as reported by Assadian *et al.* (2005) and Ogunmwonyi *et al.* (2008). Samples were also taken from 30 – 45 cm to assess the numbers of microorganisms that may have been translocated and accumulated at this depth. Sample beds from the different treatment sites were randomly chosen and samples collected from different soil depths (i.e. 0 – 30 cm and 30 – 45 cm), using a 60 mm diameter soil auger and sterile spatulas. Composite sampling is reported by Williams and Gray as a strategy (1973) to keep the error of estimates at reasonable limits. However US EPA (2005) mentions the United States' *National Environment Protection (Assessment of Site Contamination) Measure 1999* (NEPM), which specifies that composite sampling should not be used for site-specific health and ecological risk assessments. Their argument is that uncertainties in the data make this technique unsuitable for the quantitative assessment of site contamination. Discrete sampling was therefore adopted in this study, following some of the reasons suggested by the US EPA (2005) guideline on sampling.

The samples collected from the different treatment plots were placed in sterile plastic receptacles and immediately transported to the laboratory for Total Coliform (TC), Faecal Coliform (FC) and helminths egg analysis. Samples were placed in another polythene bag to retain humidity, and were kept refrigerated (4^{o}C) until used within 24 hours. Sampling was done weekly over a period of 105 days from Dec 2008 to April 2009. A total number of 90 soil samples were taken over the study period.

3.3.2 *Sampling of irrigation water*

Sampling of irrigation water was carried out between 08:00 and 10:00 hours in the morning at the time when farmers were irrigating (APHA-AWWA-WEF, 2001). The irrigation water source was agitated during this period. Sterilized 200 ml glass bottles were used to collect water from three different points in the well and at 20-m intervals along the stream. Piped water was collected directly from the water hose used by the farmers for irrgation. The samples were stored in cold icebox and transported to the laboratory for TC, FC analyses. The samples were analysed within 24 hours without loss of cool storage. Two-litre samples were taken for helminth analysis. Fifteen samples were taken weekly between Dec 2008 and April 2009.

3.4 Laboratory procedures

3.4.1 *Sterilization of equipment and material*

To avoid microbial contamination, materials used for microbiological analysis were sterilized under laboratory conditions using standard procedures. Petri dishes and test tubes were initially washed with soap and rinsed with water before allowing them to be air-dried, and sterilized.

To prevent contamination of the media used, all inoculum transfers were made aseptically in the inoculation room. The opening ends of all test tubes containing media and samples were sterilized using a naked flame before and after inoculum transfers. The inoculation loops were flamed until they turned bright red before and after each inoculation.

Glassware were sterilized by either putting them in a canister or wrapped with aluminium foil, and put in an oven at 160 ^{o}C for three hours, after washing and air-drying.

3.4.2 *Media preparation*

Liquid media

A quantity of 25.5 g of peptone Broth (MAERCK® KgaA 64271 Darmstadt, Germany) and 40 g of MacConkey Broth (OXOID® Basingstoke, Hampshire, England) were dissolved in 1 litre distilled water and heated to help dissolution. Five millilitres of the broth medium were distributed into test tubes fitted with Durham tubes. Test tubes containing media were sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool before use.

Solid media

All solid media were prepared from dehydrated stocks according to the manufacturer's instructions. All media were prepared with double distilled water, and pH adjustments were made by using a pH meter (WTW Wissenschanftlich-Techniche Werstatten, Germany).

Different growth media were prepared by dissolving 47 g of MacConkey and 23 g of nutrient agar (MAERCK®) in 1 litre of distilled water respectively. Each of the prepared media was boiled to dissolve completely. The media prepared was sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool to about 50^{o}C. About 20 ml volumes of the liquefied agar was poured aseptically into sterilized petri dishes and allowed to gel before inoculation.

Nutrient agar is a general purpose medium while MacConkey agar is a selective and differentiation medium for the detection of Enterobacteriaceae. The latter enable lactose- and/or sucrose-fermenting microorganisms to be differentiated from non-fermenting microorganisms.

3.4.3 *Determination of pH, temperature and electrical conductivity*

The pH of soil and water were simultaneously measured using pH/Cond 340i /SET (WTW Wissenschanftlich-Techniche Werstatten, Germany). The pH electrodes were calibrated before every set of measurements by using any one (single-point calibration) of the WTW technical buffer solutions (pH values at 25^{o}C:2.00 / 4.01 / 7.00 / 10.01). These buffer solutions are automatically recognized by the measuring instrument. After the calibration, the electrode was thoroughly rinsed with deionised water before the sample measurements were taken.

Fifteen grams of soil samples were mixed with fifteen millimeters distilled water and allowed to equilibrate for thirty minutes before the pH was measured (Fierer *et al.*, 2003).

3.5 Microbial examination

Total and faecal coliform populations in soil and water samples were determined using the Most Probable Number (MPN) technique (APHA-AWWA-WEF, 2001). On the other hand, the quantification method (Scwartsbrod, 1998), were used to determine the helminth egg populations in the soil and water samples. Total coliform (TC) and faecal coliform (FC) counts are necessary to indicate pollution. However, FC count is a better indication of pollution since TC count may include strains that are not associated with faecal matter (Hespanol and Prost, 1994). Helminth eggs present high risk to human and animal health (Shuval *et al.,* 1986b). Hence, helminth egg analysis is very important to measure the associated hazards with the irrigation practice.

3.5.1 *Total and faecal coliform population estimations in soil samples from irrigated fields and irrigation water*

Quantitative analyses were performed on irrigation water and soil samples for total and faecal coliforms. For coliform counts, ten grams (dry weight) of each soil sample was weighed into 90 ml of distilled water buffered with NaCl (10 g/L). The mixture sample was pulsified for three minutes. Further tenfold serial dilutions were made and triplicate tubes of MacConkey broth supplied by MAERCK® (Germany) were inoculated from each dilution. Irrigation water samples were also serially diluted before inoculation and incubated at 37^{o}C for total coliforms and 44.5^{o}C for faecal coliforms for 24 to 48 hours (APHA-AWWA-WEF, 2001). Positive tubes

(acid or gas production or both) were selected and the numbers of bacteria were obtained from MPN (Most Probable Number) tables (APHA-AWWA-WEF, 2001).

3.5.2 *Quantification and identification of helminth eggs in irrigation water and soil*

Helminth egg population in water and soil were determined using the flotation sedimentation method which is modified US-EPA method by Schwartzbrod (1998). Identification of specific helminth eggs was carried out using bench aids for the diagnosis of intesitinal parasites (WHO, 1994).

The reagents used include ZnSO4, acid/alcohol buffer and ethyl acetate. These were prepared as follows: 1) 573 g of zinc sulphate (Harris reagent; Philip Harris plc, Shenstone, England) was dissolved completely in one litre of sterilized deionized water to produce zinc sulphate solution of specific gravity of about 1.2, and (2) Acid/alcohol buffer solution was prepared by adding 5.16 ml H_2SO_4 to 350 ml of ethanol. Sufficient deionized water was then added to the acid/alcohol mixture to produce 1 litre of the solution.

3.5.2.1 *Identifying and quantifying helminth in soil*

Methods of estimating populations of bacteria and helminth eggs in soil usually involve pre-treating the samples so as to release the microorganisms into sterile diluents. Twenty gram (20 g) portion of soil samples was blended at high speed in 200 ml distilled water buffered with 5 ml of sodium-chloride solution for about 1 minute and the volume of the mixture was further increased to 2 litres. This mixture was then allowed to settle overnight to enable the helminth eggs settle under their own weight. As much of the supernatant as possible was sucked up and the sediment

transferred into 15 ml centrifuge tubes. The 2-litre containers were rinsed 2-3 times with deionized water and the rinses were transferred into centrifuge tubes. The tubes were then centrifuged at 1450 rpm for three minutes. The sediments in the centrifuge tubes for each sample were pooled into one centrifuge and centrifuged again at 1450 rpm for three minutes.

The supernatant was poured away and the deposit was re-suspended in about 150 ml ZnSO4 (372 g/l) of density 1.3. The mixture was homogenized with a spatula and centrifuged at 1450 rpm. At a density of 1.3 ($ZnSO_4$), all helminth eggs float leaving other sediments at the bottom of the centrifuge tube. The $ZnSO_4$ supernatant (containing the eggs) was poured into a 2-ltire flask and diluted with at least one litre of water. This was allowed to settle overnight for the eggs to settle again. As much supernatant as possible was sucked up and the deposit was re-suspended by shaking. The resuspended deposit was put into centrifuge tubes. The 2-litre container was rinsed 2-3 times with deionised water and the rinsed water added to the centrifuged tubes and centrifuged at 1600 rpm for three minutes. The deposit was pooled into one tube and centrifuged again at the same speed and for the same period of time.

Thereafter, the deposit was re-suspended in acid/alcohol (H_2SO_4 + C_2H_5OH), after sucking much of the supernatant, and ethyl acetate was added. The mixture was shaken and the centrifuge tube occasionally opened to let out gas before centrifuged at aqueous phase representing the ethyl ether and acid/alcohol, respectively solution was formed. With a micropipette, as much of the supernatant as possible (starting from the lipophilic and then the aqueous phase) was sucked up leaving about 1 ml of deposit. The deposit was observed on a Sedgwick-Rafter cell under the microscope (x100) and the eggs counted. The number of helminth eggs was expressed per gram soil (oven-dried weight basis).

3.5.2.2 *Identify and quantifying helminth in irrigation water*

Irrigation water samples were allowed to settle overnight. This was to enable the helminth eggs settle under their own weight. Much of the supernatant as possible was sucked up and the sediment transferred into 15 ml centrifuge tubes. The 2-litre containers were rinsed 2-3 times with deionized water and the rinses were transferred into centrifuge tubes. The tubes were then centrifuged at 1450 rpm for three minutes. The sediments in the centrifuge tubes for each sample were pooled into one centrifuge and centrifuged again at 1450 rpm for three minutes.

The supernatant was poured away and the deposit was re-suspended in about 150 ml ZnSO4 (372 g/l) of density 1.3. The mixture was homogenized with a spatula and centrifuged at 1450 rpm. At a density of 1.3 ($ZnSO_4$), all helminth eggs float leaving other sediments at the bottom of the centrifuge tube. The $ZnSO_4$ supernatant (containing the eggs) was poured into a 2-ltire flask and diluted with at least one litre of water. This was allowed to settle overnight for the eggs to settle again. As much supernatant as possible was sucked up and the deposit was re-suspended by shaking. The resuspended deposit was put into centrifuge tubes. The 2-litre container was rinsed 2-3 times with deionised water and the rinsed water added to the centrifuged tubes and centrifuged at 1600 rpm for three minutes. The deposit was pooled into one tube and centrifuged again at the same speed and for the same period of time.

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aqueous phase) was sucked up leaving about 1 ml of deposit. The deposit was observed on a Sedgwick-Rafter cell under the microscope (x100) and the eggs counted. The number of helminth eggs was expressed per litre of irrigation water samples.

3.6 Dry weight determination

The total number of coliform and helminth eggs in soils is usually expressed in soil dry weight basis. To achieve this, a known weight of soil sample was placed in glass dishes and left to dry in a hot air oven at 105^{o}C for 24 hours. After drying, the samples were placed in a desiccator and allowed to cool before re-weighing. Mean dry weight was calculated and faecal coliform levels in soil expressed in dry weight. The difference in weight after drying the soil sample was also taken as the soil moisture weight.

3.7 Soil particle size analysis

This was done using the hydrometer method (Gee and Bauder, 1986). Reagents used for this experiment included 5% sodium hexametaphosphate (calgon) solution, 30% Hydrogen peroxide (H_2O_2), distilled water and amyl alcohol. Equipments used included the mechanical shaker, sedimentation cylinder tube, stop clock, thermometer, hydrometer, and a 1000 ml screw lid bottle for shaking.

For each sample, 51 g of air-dried soil was weighed into a one-liter screw lid shaking bottle. 100 ml of the distilled water was added and the mixture swirled to wet soil thoroughly, after which 20 ml of H_2O_2 was added. H_2O_2 destroys soil organic matter and hence frees the individual

classes of soil. Fifty millimeters of 5% sodium hexametaphosphate solution was then added to the mixture. To minimise foaming, two drops of amyl alcohol were added to the mixture and gently swirled to mix thoroughly. The mixture was then shaken on a mechanical shaker to about 2 hours and the contents were transferred to a 1000 ml sedimentation cylinder. Water washings of all soil particles were added to the sedimentation tube. Distilled water was then added to make up to the 1000 ml mark. After 40 seconds, the first hydrometer and temperature (with the help of the thermometer) readings were taken. The sample was then allowed to stand undisturbed for three hours. After this, the second hydrometer and temperature readings were taken.

The following calculations were made to determine the soil textural classes:

$\%\ \text{Sand} = 100 - [H_1 + 0.2\ (T_1 - 20) - 2] \times 2$

$\%\ \text{Clay} = [H_1 + 0.2\ (T_2 - 20) - 2] \times 2$

$\%\ \text{Silt} = 100 - (\%\ \text{Sand} + \text{clay})$

Where:

H_1 = 1st Hydrometer reading at 40 seconds

T_1 = 1st Temperature reading at 40 seconds

H_2 = 2nd Hydrometer reading at 3 hours

T_2 = 2nd Temperature reading at 3 hours

– 2 = Salt correction to be added to hydrometer reading

0.2 (T – 20) = Temperature correction to be added to hydrometer reading, and

T = degree celcius

The hydrometer was removed and wiped down after each reading.

3.8 Characterization of bacteria in soils irrigated with water from different sources

The best practice of characterizing species from samples is preceded by isolation of pure cultures (Holt, 1986). Samples were collected during the latter sampling days were used in this study. Identification of bacteria was aided by the Laboratory manual to accompany microorganisms in our world (Atlas *et al.*, 1995). Preliminary tests (presumptive and confirmatory) tests were used to determine the Genera of bacteria present in each soil sample.

1 g of each sample was inoculated into sterile tubes containing peptone water. The tubes were then covered tightly with cotton plugs and incubated overnight at 37^{o}C. From a tube showing positive growth, a drop of the inoculated peptone water was put on glass slide using a sterile Pasteur pipette and covered with a cover slip for microscopy. Thereafter, the prepared specimen was viewed under the microscope and observations were recorded.

Also, a loopful of the supernatant from the inoculated tubes was taken with a sterile copper loop and streaked on nutrient agar in sterile, covered, glass plates and incubated overnight at 37^{o}C. MacConkey agar plates were also inoculated using the fresh overnight cultures. The inoculated agar plates were examined to ascertain the particular bacteria present using differing characteristics such as size, odour, shape, colour, and lactose fermentation. Each colony type was subjected to Gram staining after it had been examined for special growth characteristics. For *Escherichia coli,* two drops of Kovac's reagent were added to overnight peptone broth to establish its presence or absence.

3.8.1 Gram staining of bacteria cultures

Gram staining is used to differentiate bacterial species into two large groups (Gram negative and Gram positive) based on the chemical and physical properties of their cell walls. A loopful of the cutlture under study was transferred onto the surface of a clean glass slide. After, the slide was flooded with crystal violet solution for up to one minute and washed off briefly (not over 5 seconds) with a gentle jet of tap water. After draining the smeared slide, it was again flooded with Gram iodine solution, and allowed to act (as a mordant) for one minute. This was also washed under running tap water and drained. Excess water on the slide was blotted out so that alcohol used for decolorization is not diluted. Stained films were flooded with 95% alcohol for 10 seconds and washed off with water. The slide was drained afterwards, flooded again with safranin solution and allowed to counterstain for 20 – 30 seconds. The sufranin-flooded slide was washed with tap water, drained and blotted with filter paper. The slides were then examined under the oil immersion lens.

3.9 Statistical analysis of data

The results were analysed using SPSS for Windows 16.0.1 (SPSS Inc., Chicago IL, USA) and Microsoft Office Excel 2007 (Microsoft Corporation).

Total and faecal coliform populations (MPN) were normalised by log transformation before analysis of variance (ANOVA). ANOVA was used to compare the total and faecal coliform levels, as well as helminth eggs in the different treatment plots. The t-test (one sample and two

independent samples) was used to test significance of difference between mean faecal coliform levels on different irrigation treatment plots and in irrigation water from different sources.

The mean, standard errors and variance were calculated using the Microsoft Office Excel 2007 and SPSS for Windows 16.0.1 (SPSS Inc., Chicago IL, USA). Unless otherwise stated, results of analysis are at $p < 0.05$ level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Microbiological quality of irrigation water

4.1.1 *Total and faecal coliform levels in irrigation water*

The total coliform levels observed in the irrigation water sources ranged between 7.5 x 10^4 and 7.5 x 10^9 (Fig 4.1.1) and those of the faecal coliform ranged between 1.5 x 10^3 and 2.3 x 10^7 (Fig 4.1.1).

Table 4.1.1 Mean total and faecal coliform bacteria in irrigation water at vegetable production site at Atonsu

Irrigation water source	Log_{10} total and faecal coliform levels (MPN[a]) / 100 ml			pH
		Total Coliform	Faecal Coliform	
Well	Range (N = 90)	4.88 – 7.97	3.17 – 5.88	6.4 – 7.5
	Geometric Mean	6.05 (±0.93)[b]	4.07 (±0.79)	7.0 (±0.08)
Stream	Range (N = 90)	6.04 – 9.88	4.32 – 7.36	6.9 – 7.6
	Geometric Mean	7.59 (±1.11)	5.39 (±0.94)	7.3 (±0.04)
Piped Water	Range (N = 90)	0.00 – 0.72	0.00 – 0.00	7.00
	Geometric Mean	0.43 (±0.23)	0.00 (±0.00)	7.00 ((±0.00)

[a]Most Probable Number
[b]Figures in parenthesis are the standard errors

Mean total and faecal coliform levels in stream water were significantly ($P < 0.05$) higher than those of the well and piped water sources. Samples from well and stream water exceeded the WHO (1989) recommended level of 1000 faecal coliform per 100 ml. However, piped water samples showed very low coliform levels with total coliform ranging between 0.03×10^0 and 7.2×10^1. Faecal coliform were absent in the piped water samples that were taken from the vegetable production site.

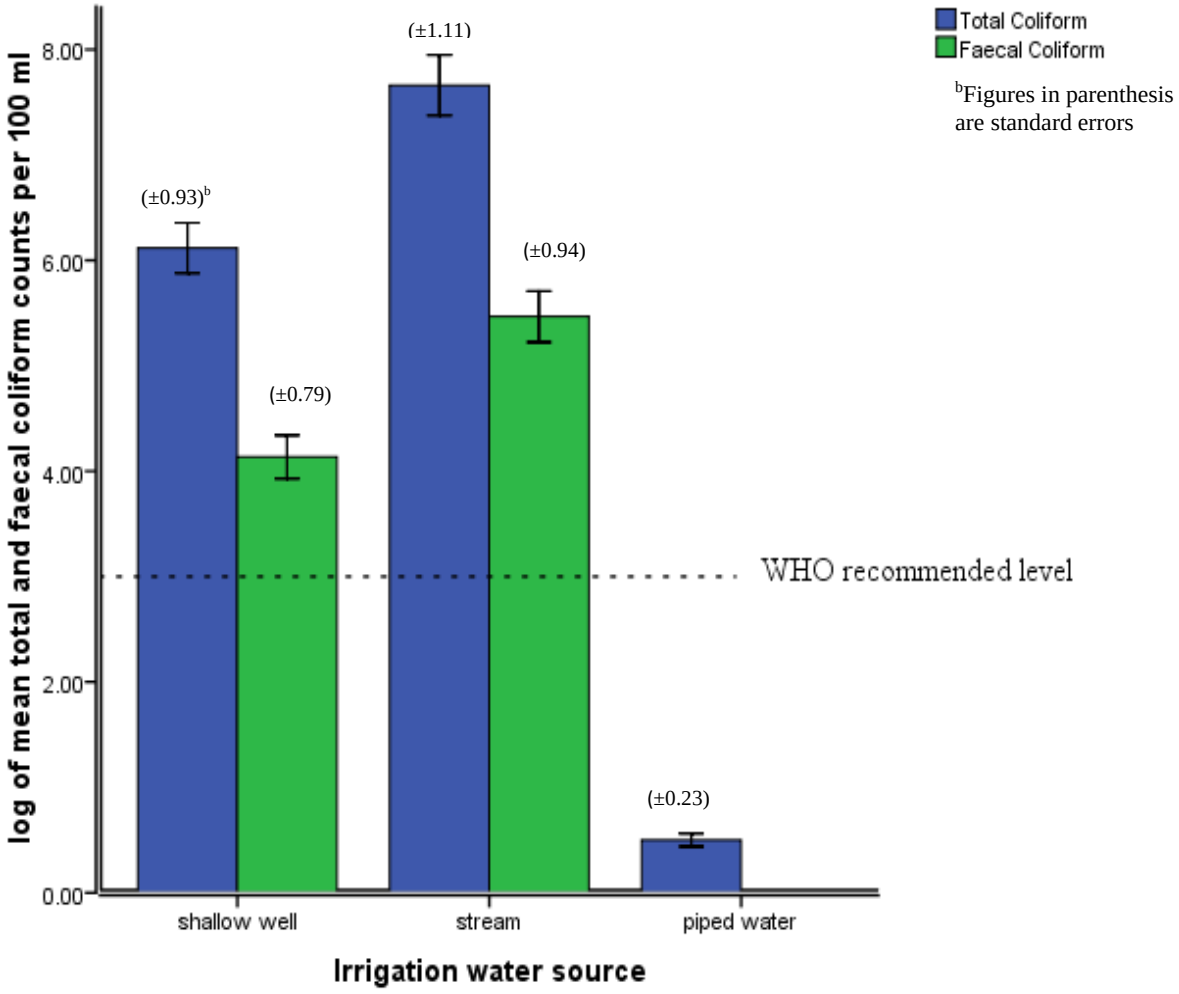


Fig 4.1.1 Total and faecal coliform levels in irrigation water from shallow dug out wells, stream and stand pipe.

4.1.2 *Helminth egg population in irrigation water*

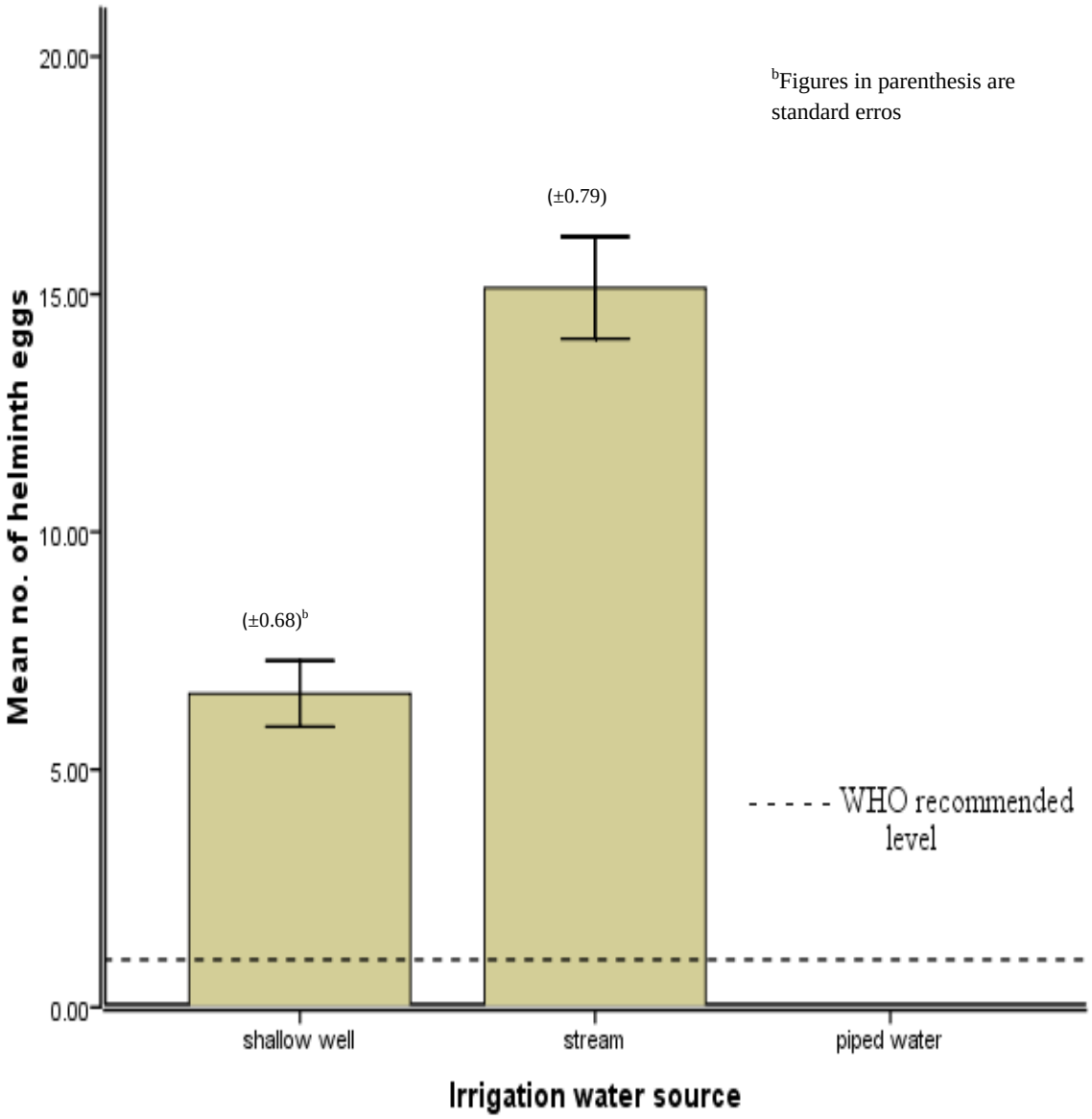
A number of different types of helminth eggs were identified from all irrigation water sources except piped water. These included the eggs of *Ascaris lumbricoides, Strongyloides stercoralis, Fasciola hepatica* and *Schistosoma species* (Table 4.1.2).

Significantly higher numbers of helminth eggs were recorded in the stream samples than those from the shallow dug out wells. No helminth eggs were recorded in piped water (Fig 4.1.2). Samples of irrigation water from well and stream exceeded the WHO (1989) recommended level of 1 egg per liter.

Table 4.1.2 Mean number of helminth eggs in irrigation water at vegetable production site in Atonsu

	Mean numbers of helminth eggs (l^{-1})	
Helminth	Shallow well	Stream
Ascaris lumbricoides	4 (±0.60)	5 (±0.65)
Strongiloides stercoralis	3 (±0.47)	7 (±0.77)
Fasciola hepatica	1 (±0.00)	3 (0.22)
Schistosoma species	2 (±0.16)	3 (±0.49)

Figures in brackets are standard errors



[b]Figures in parenthesis are standard erros

Figure 4.1.2 Helminth egg populations (per litre) in irrigation water sources at vegetable growing site

4.2 Contamination levels of soils of wastewater irrigated fields

Total coliform counts in the three plots ranged from 0.03 and 2.1 x 10^6 (Table 4.2.1). Also, faecal coliform populations recorded ranged from 0.03 and 9.5 x 10^4 (Table 4.2.1). The lower values were recorded from the control plots while the wastewater-irrigated plots recorded higher values (Figure 4.2.1). Similarly, helminth eggs recorded ranged from 0 and 30 eggs (Table 4.2.1). The control plots and wastewater-irrigated plots recorded the lowest and highest values respectively, from the study (Figure 4.2.1).

4.2.1 *Distribution of coliforms and helminth eggs in the soil*

The microorganisms identified in the study were detected in both the upper soil profile (0 – 30 cm) and the lower soil profile (30 – 45 cm). The upper soil profile had higher counts of coliforms and helminth eggs than the deeper soil profile (Table 4.2.1, Figure 4.2.1 and Figure 4.2.2). Wastewater irrigated plots had higher numbers of coliforms and helminth counts than those obtained for the potable water irrigated and no irrigation plots (Table 4.2.1 and Figure 4.2.1). The lowest coliform and helminth counts were recorded from the no irrigation plots at (Table 4.2.1).

There were significant differences ($P \leq 0.05$) in the number of coliforms and helminth eggs in soils sampled at a depth of 0 – 30 cm from the different treatment plots (Appendix 3A). The wastewater irrigated plots had higher coliforms and helminth egg populations than those of the potable water irrigated and no irrigated plots (Table 4.2.1). In addition, the analysis showed that

significant differences ($P \leq 0.05$) existed in helminths counts between soils from potable water irrigated, wastewater irrigated, and no irrigation beds (Appendix 3A).

Table 4.2.1: Microbial population distribution in the soil profile of wastewater irrigated vegetable farms

Soil Depth (cm)	Irrigation treatment	Log_{10} Geometric mean (MPN) / 10 g		Mean Helminths no. per 10 g
		T.C	F.C	
	Potable water irrigation	4.62 (±0.19)	3.77 (±0.18)	12(±1.55)
0 – 30	Wastewater irrigation	5.10 (±0.16)	4.06 (±0.15)	20(±11)
	No irrigation	1.66 (±0.25)	0.76 (±0.22)	1(±0.19)
	Potable water irrigated soil	2.97 (±0.13)	1.95 (±0.14)	1(±0.31)
30-45	Wastewater irrigated soil	3.40 (±0.14)	2.53 (±0.17)	4 (±1.22)
	No irrigation	0.72 (±0.19)	< 0.03	0 (±0.00)

Figures in bracket are standard errors
T.C = Total coliforms
F.C= Faecal coliforms

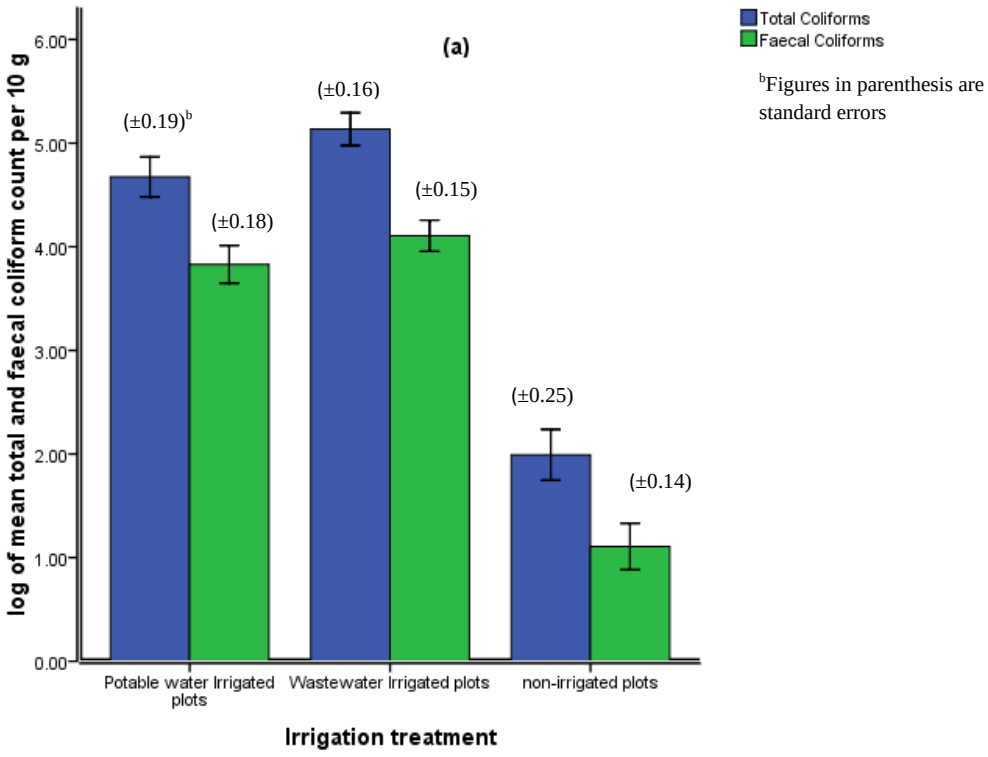
At the 30 – 45 cm depth, significant differences ($P \leq 0.05$) were recorded for the number of coliforms and helminths eggs (Appendix 4A). Also, differences in faecal coliform counts between potable water, wastewater irrigated, and no irrigated plots were significant at $P = 0.012$.

However, helminth counts recorded from potable water and no irrigation plots showed no significant differences (P = 0.440) (Appendix 4A).

Table 4.2.2 Helminth levels in soils from different depths on different treatment plots at vegetable production site at Atonsu

		Mean numbers of helminth 10 g^{-1}			
Depth (cm)	Plots	*Ascaris lumbricoides*	*Strongyloides stercoralis*	*Fasciola hepatica*	*Shistosoma species*
0 - 30	Potable water irrigated	6 (±3.7)	4 (±3.5)	2 (±0.9)	1 (±2.0)
	Wastewater irrigated	10 (±5.4)	4 (±2.5)	1 (±2.0)	2 (±1.8)
	No irrigation	0 (±0.0)	0 (±0.6)	0 (±0.0)	0 (±0.0)
30 - 45	Potable water irrigated	0 (±0.7)	1 (±1.0)	0 (±0.0)	0 (±0.0)
	Wastewater irrigated	3 (±3.9)	1 (±1.8)	1 (±0.6)	1 (±0.0)
	No irrigation	0 (±0.0)	0 (±0.0)	0 (±0.0)	0 (±0.0)

Figures in parenthesis are standard deviation: values are rounded off to the one decimal place



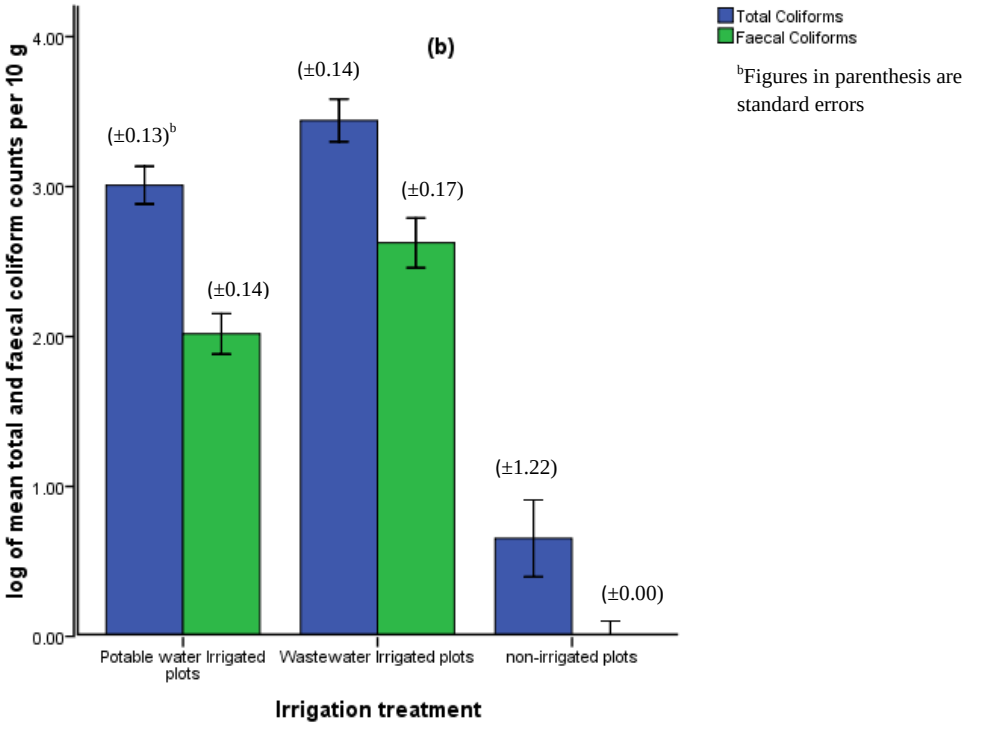


Fig 4.2.1 Total and faecal coliform counts of soil samples from wastewater irrigated vegetable farms at Atonsu, Kumasi. a) at depth of 0 -30 cm, and b) at depth of 30 – 45 cm.

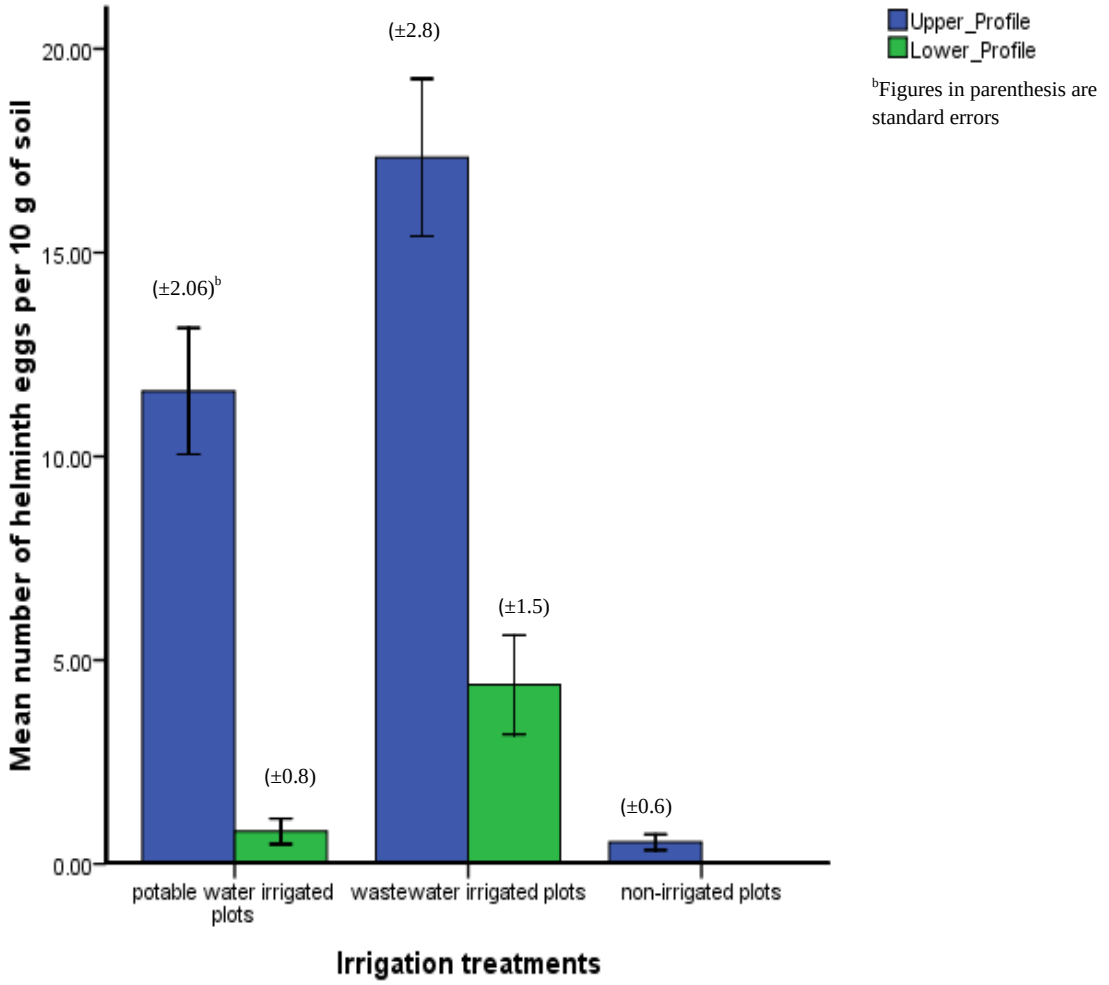


Fig 4.2.2 Helminth egg counts of soil wastewater irrigated vegetable farms at Atonsu at upper (0 -30 cm) and lower (30 – 45 cm) soil profiles.

4.3 Physical parameters of soils from fields irrigated with different quality of irrigation water

4.3.1 Soil texture

Particle size analysis (PSA) showed that soils sampled were either sandy or loamy sand. Sand was the most predominant of all soil samples, always constituting more than 80% (Table

4.3.1.1). All top soil samples from the study area were generally loamy sand, whiles those from the 30 – 45 cm depth were sandy (Figure 4.3.1.1).

Table 4.3.1.1: Textural class of soils along the profile of different irrigated beds

Sample identification		Percentage (%)			Texture
Depth/cm	Soil treatment	Sand	Clay	Silt	
0 - 30	Potable Water	82.4	10.0	7.6	Loamy sand
	Wastewater	82.4	12.0	5.6	Loamy sand
	No irrigation	82.4	12.0	5.6	Loamy sand
30 - 45	Potable Water	92.4	4.0	3.6	Sand
	Wastewater	92.4	4.0	3.6	Sand
	No irrigation	90.4	6.0	3.6	Sand

4.3.2 Soil pH and moisture

Soil pH was higher (approximately 0.2 units) in wastewater-irrigated fields than in the control fields (Table 4.3.2.1). Also, the potable-water irrigated fields had higher soil pH than the control fields. This trend was similar for both soil depths.

Soil moisture varied significantly among the different treatment fields and also at different soil depths (Appendix 2A). Soil moisture was higher in the wastewater-irrigated fields than in the control fields (Table 4.3.2.1).

Soil pH and moisture increased with increasing depth in all fields at all depths (Table 4.3.2.1).

Table 4.3.2.1: Physical quality of soils from beds with different treatments

Soil depth (cm)	Soil treatment	Mean pH	Mean soil moisture (g/ 10g of soil)
0 – 30	Potable water irrigated soil	6.57 (±0.03)	1.51 (±0.04)
	Wastewater irrigated soil	6.70 (±0.03)	1.71 (±0.04)
	No irrigation	6.49 (±0.11)	0.90 (±0.06)
30 – 45	Potable water irrigated soil	6.75 (±0.07)	0.98 (±0.03)
	Wastewater irrigated soil	6.80 (±0.06)	1.49 (±0.03)
	No irrigation	6.62 (±0.07)	0.65 (±0.03)

Figures in bracket are standard errors

4.4 Characterization of helminthes and bacteria in sampled soils

4.4.1 *Helminth eggs*

Helminth eggs were identified using the WHO (1994) Bench Aid for the diagnosis of intestinal parasites. *Ascaris lumbricoides* were identified by their brown elongated cells and larger sizes (approximately 90 μm by 45 μm). *Strongiloides stercoralis* were identified by their attenuated tails, prominent genital primodium and elongated structure (approximately 350 μm by 15 μm). *Fasciola hepatica* were identified by their operculum at one end, regular eclipse and thin shell. *Schistosoma* spp. were identified by their large elongated (approximately 120 μm by 50 μm) structure and terminal spine (*Schistosoma haematobium*), tapered and rounded anterior end and possessing a lateral projection at the posterior end (*Schistosoma mansoni*).

Relatively, Ascaris were recorded as contributing the highest counts at both soil depths in the different plots (Table 4.2.2). In decreasing numbers, the next higher counts were the *Strongyloides stercoralis* followed by the *Fasciola hepatica* and *Shistosoma* species (Table 4.2.2). The numbers of each type of helminth egg identified decreased with increasing depth (Table 4.2.2).

4.4.2 *Characterization of bacteria in wastewater irrigated soils*

All soil samples had the following bacteria genera observed in them through preliminary identification: *Escherichia, Bacillus, Staphyloccus, Pseudomonas* and *Clostridium.*

Following incubation after 24 – 48 hours, the inoculated nutrient and McConkey agar plates were examined for typical colonial morphology. The peptone water in the plain tubes also had a

cloudy appearance indicating the presence of bacteria (Atlas *et al.*, 1995). The Gram reaction of one type of bacteria colony showed Gram-positive cocci in clusters. These colonies were slightly raised, non-mucoid, round and shiny on the nutrient agar, appeared pink (lactose fermentor) on McConkey agar and were therefore predicted to be *Staphylococcus* (Atlas *et al.*, 1995). *Pseudomonas* were identified as Gram negative rods after they had been stained by the Gram technique (Atlas *et al.*, 1995; Holt, 1986). They were non mucoid, slightly raised, shiny, translucent and pale coloured on McConkey agar (Atlas *et al.*, 1995).

Some other bacteria colonies showed Gram positive single rods. These colonies which were predicted to be *Clostridium* were round, non-mucoid, grey and opaque on nutrient agar. *Bacillus* colonies appeared as Gram positive large rods with stained capsules (Atlas *et al.*, 1995). These colonies were large and irregular with wavy edges and a grey colour (Atlas *et al.*, 1995; Holt, 1986). There were also a number of colonies which were Gram negative rods. On account of these colonies being slightly raised, non-mucoid and grey on nutrient agar as well as opaque and pink on McConkey agar, they were thought to be produced by bacteria genera of the family Enterobacteriaceae (Atlas *et al.*, 1995).

The presence of an indole ring (pink color) was observed on the surface of the peptone broth, confirming the presence of *Escherichia coli.*

CHAPTER FIVE

5.0 DISCUSSION

5.1 Microbial quality of irrigation water

Microbial quality of irrigation water from the dugout wells and stream observed in this study supports the earlier reports of the usage of poor quality irrigation water for urban vegetable production in Ghanaian cities (Keraita and Drechsel, 2004; Cornish *et al.*, 2001; Mensah *et al.*, 2001; Keraita *et al.*, 2003; Cornish *et al.*, 1999). The range of total and faecal coliform observed confirms Keraita and Drechsel's (2004) report that faecal coliforms typically reached values of 10^6–10^8/100 ml while total coliform levels often range from 10^8–10^{10}/100 ml of irrigation water in Kumasi.

The observation that microbial contamination levels were significantly different between the well and stream water confirms report of Amoah *et al* (2005) that on site streams used as irrigation water source may have higher contamination levels than on site hand-dug shallow wells. This may suggest that the shallow wells may pose relatively less risk to farmers and consumers (Amoah *et al*, 2005). On the other hand, Cornish *et al* (1999) recorded in Kumasi temporarily higher faecal coliform population in shallow wells than in nearby streams. Amoah and his colleagues argued that the difference may be attributed to the shallower wells used in the latter's study which got more easily contaminated through surface runoff from the field.

In addition, some observations from this study may account for the significantly higher microbial contamination levels in streams than wells on the farming site. Observations include farmers entering the stream with their working boots to fetch water. These boots carry soil and poultry

manure particles that may contain microorganisms which may eventually contaminate the stream. Another observation was the usage of the stream by nearby inhabitants for their domestic purposes. Such users also, after using pathways contaminated with manure, enter the stream with various contaminated objects (for e.g. footwears, containers etc). Apart from surface run-offs, there may also be a possible seepage from the farming fields (non-point source) into the stream.

5.2 Contamination levels of soils irrigated with wastewater

5.2.1 Comparison of pathogen levels in wastewater irrigated fields and control fields

The presence of fecal coliform in a drinking water sample often indicates recent fecal contamination – meaning that there is a greater risk that pathogens are present (Toze, 1997). The level of soil contamination recorded in the study raises concerns when considering the potential environmental and health risks exposed to farmers and consumers of the crops cultivated in the study area. Shuval *et al.* (1986a) showed that an outbreak which occurred as a result of vegetables irrigated with wastewater was linked with *Vibrio cholera* present in the irrigated soils. Geophagia, as well as involuntary ingestion by farmers and their children could expose them to a high risk (Lagoy 1987; Toranzos and Marcus, 1997). As the vegetables frequently come in contact with the soils, they become contaminated with soil enteric pathogens, eventually posing health risks to the consumers of these farm products (Santamaria and Toranzos, 2003). The use of the wastewater irrigation sources (dug-out wells and stream), as well as the manure as fertilizer, are potential sources of pathogens in the soils (Amoah *et al.*, 2005; Beuchat, 2002).

In this study, wastewater irrigated fields had comparatively higher coliform and helminth counts than the control fields (i.e. potable irrigated fields and no irrigated fields) (Table 4.2.1). Wastewater application to soil generally raises activity of soil microorganisms by increasing soil organic matter (Toze, 1997; Goyal *et al.,* 1995). Studies show the presence of organic matter extends the survival of total and faecal coliforms, and helminth eggs (Malkawi and Mohammed, 2003; Toze, 1997; Tate, 1978). Yadav *et al* (2002) in their studies observed that sewage irrigated soils had higher organic content than that of soils irrigated with tubewell water and/or occasionally with sewage water. Wastewater is carrier of bacteria, viruses, protozoa and nematodes. These microorganisms are transferred into the soil when wastewater is applied to the soil (Kalavrouziotis *et al.*, 2008). Potable water, however, did not contain faecal coliforms and helminth eggs and thus its application will most likely not introduce pathogenic microorganisms in the soil.

Pathogen movement in the soil may be facilitated by the physical conditions and the composition of the soil. The higher percentages of sand in the soils of our experimental fields enhance permeability, hence promoting the movement of pathogens deep into soil (Sinton, 1986; Abu-Ashour *et al.*, 1994). Bacteria can move through soils to great depths (Eliot, 2002). Romero (1970) reported that after two days, fecal coliform and fecal streptococcus organisms were observed to travel over 500 m (1500 ft) after the application of tertiary treated wastewater to coarse gravel soils. Soils facilitating deep penetration include sand, sandy gravel, and gravel (Eliot, 2002).

5.2.2 Distribution of coliforms and helminth eggs in the soil

The higher numbers of coliform bacteria and helminthes in the 0 – 30 cm soil profile than those of the 30 – 45 cm could be attributed to many factors. For example, the organic matter and nutrient contents in soils decline with increasing depth (Yadav *et al.*, 2002; Fierer *et al.*, 2003). Thus less microbial activities such as growth and reproduction will take place at the lower levels. The sieving effect of soil, which is influenced by particle size, texture (i.e., sandy, loamy, clayey), and adsorption, can greatly reduce bacterial movement (Eliot, 2002; Krone, 1968; Malkawi and Mohammed, 2003).

Also, a number of studies have indicated that there is a rapid die-away or removal of both bacterial indicator organisms and of pathogenic bacteria and viruses in wastewater-irrigated soil. Various reports indicate that as much as 5-log reduction in 2 days can occur under field conditions (Fattal *et al.*, 2004; Bergner-Rabinowitz, 1956; Rudolfs *et al.*, 1951; Sadovski *et al.*, 1978; Armon *et al.*, 1995). The cumulative effect of fewer microorganisms reaching the deep soils (as a result of the sieving effect of soil) and rapid pathogenic die-away may result in the lower microbial counts at the lower depths observed in this study.

Another reason for lower microbial counts in the deep soils could be the lack of substrate diversity at these depths (Fierer *et al.*, 2003). Just below the surface, substrate diversity increases, with inputs of litter, root exudates, root litter, soil organic matter (SOM) etc. However, there is reduction in substrate diversity and availability of compounds below 20 cm (Fierer *et al.*, 2003). The quantity and quality of carbon substrates decline with progressively deeper layers in the soil profile (Fierer *et al.*, 2003). Surface soils are rich in available carbon substrates from the input of root exudates, surface litter and root detritus (Fierer *et al.*, 2003). In contrast, the rates of

carbon input to the lower horizons are generally low and the carbon tends to be of limited lability (Richter and Markewitz, 1995; Ajwa *et al.,* 1998; Trumbore, 2000; Fierer *et al.,* 2003). Pathogenic microorganisms living between 30 – 45 cm will therefore die-off more rapidly than those living just between 0 – 30 cm depth.

Soil moisture favours growth of pathogenic microorganisms in soil (Santamaria and Toranzos, 2003). Salvato *et al.* (2003) carried out studies which showed that soil moisture of about 10 to 20 percent of saturation appears to be best for survival. The result of the study show that soil moisture content was relatively higher in the wastewater irrigated fields than the PW-irrigated fields. This observation could be due to the higher amount of clay in the wastewater irrigated soils than that of the PW-irrigated soils (Table 4.3.1.1). Clay has a higher moisture holding capacity than sand. Previous work has shown that variability in soil moisture can influence microbial populations (Kieft *et al.,* 1993; Schimel *et al.,* 1999). The wastewater irrigated soils at the top profile are thus able to hold more water than that of the PW-irrigated soils.

In addition, this study showed that there was generally higher percentage of clay in soils from the upper soil profile than those from the lower profiles (Table 4.3.1.1). The sorption of bacterial cells to clay has been demonstrated to be advantageous to their survival (Toze, 1997). Roper and Marshall (1978) showed that the presence of clay in soils significantly reduced predation. Clays protect microbial cells by creating a barrier between them and microbial predators and parasites (Toze, 1997). Thus, in our study, lower clay content in the lower soil profile could have also contributed to the increased die-off rates of microorganisms at these depths.

Table 4.3.2.1 shows higher soil pH (approximately 0.2 units) in wastewater-irrigated fields than in the control sites. Mancino and Pepper (1992) had similar results when they found that effluent

waster irrigation increased soil pH by 0.1 to 0.2 units. Increases in soil pH under land application of wastewater have also been previously reported by Jahantigh (2008), Qian and Mecham (2005) Schipper *et al.* (1996), and Pepper and Mancino (1992). However, Schipper *et al.* (1996) observed an increase in soil pH by 0.8 units after applying tertiary-treated domestic wastewater to a forest site for 3 yr at 4.9 cm wk^{-1}. Schipper and his colleagues suggested that the rise in soil pH was likely related to a high rate of denitrification that produced hydroxyl ions. Qian and Mecham (2005) also found out in their research that soil pH in wastewater-irrigated sites increased by approximately 0.3 units, and attributed the increase to higher pH and higher bicarbonate concentration in the wastewater than surface water. The higher soil pH in wastewater irrigated fields likely resulted from the higher pH of the wastewater than that of the piped water.

Soil composition and pH influence the adsorptive ability of the soil matrix. Experiments have shown that there is increased sorption at slightly acidic or neutral pH and little adsorption at pH values above 8 (Toze, 1997). Generally, increased ability to adhere to surfaces reduces the die-off rates in soils for bacteria. It also reduces predation effects and other influences such as changes in the surrounding environment (Toze, 1997). In this study, the soil pH observed were slightly acidic and thus could have promoted the survival of the soil bacteria (Wikipedia, 2009; Toze, 1997). *Escherichia coli*, for instance, exhibited greatest tolerance within the zone pH 5.0 to 6.4 (Rudolfs *et al.*, 1950). Though the pH range observed in our study is favourable to the survival of the microorganisms, soil pH is not likely to cause variations in the microbial populations along the soil profile. This is because there was no statistical difference ($P > 0.05$) in pH values from the different sampling sites of the soil (see Appendix 1A).

Helminths are ranked high risk (Shuval *et al.*, 1986b) among pathogens present in soils and wastewater. The significant differences observed in helminth counts from the different treatments may be indicative of the variety of contamination sources that the different treatment plots were exposed to. The levels of helminth contaminations observed may contribute to high infective dose to farm workers, nearby communities, and consumers of contaminated vegetable products through various exposure routes (contact, geophagia or voluntary ingestion of soil, involuntary ingestion of soil as a result of wind, consumption of contaminated vegetable products etc). Surface runoffs from these beds into streams contribute to the high level of helminth found in the irrigation source.

The results of the study show that the farm workers have a high risk of helminth infection from contact with the soil or wastewater (WHO, 2006; WHO, 1989; Toze, 1997, Blumenthal *et al.*, 2000). Voluntary (geophagia) and involuntary ingestion of soil as a result of wind could present a risk to the farmers and nearby communities to the farming area (Toranzos and Marcus, 1997; Lagoy, 1987). Nearby communities, involved in the use of the stream water for domestic purposes, also have high risk of infection. The helminth eggs identified (Table 4.2.2) are of significant health risk. WHO (2006) reports that soil-transmitted helminthes produce a wide range of symptoms including intestinal manifestations (see Section 2.3.1.3).

Such risks can be reduced, even eliminated, by the use of less-contaminating irrigation methods (e.g. localised techniques such as bucket drip kits) and by the use of appropriate protective clothing (i.e. shoes or boots for fieldworkers and gloves for crop handlers) (WHO, 2006; Pescod, 1992; Keraita *et al.*, 2007). These health protection measures are expected to have an important effect (WHO, 2006). This is especially true for wearing shoes or boots where there is a risk of

hookworm or schistosomiasis transmission. The farmers should be provided with access to sanitation facilities and adequate water for drinking and hygienic purposes in order to avoid the consumption of, and any contact with, soil and wastewater.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 CONCLUSIONS

- This study has confirmed that wastewater used in vegetable production at the study area is highly contaminated with total coliform, faecal coliform and helminth eggs. As a result, pathogens may be transferred from the wastewater into soils which are irrigated with the irrigation source. The soils could serve as source of contamination for the cultivated crops and farm workers. Nearby communities and most importantly farmers using the stream water could be at high risk of infection from the water source. Reduction of pathogenic microbes in the stream water could eventually reduce the contamination levels in the soils.

- Wastewater irrigated fields are more contaminated than the piped-water irrigated and non-irrigated fields. Wastewater is a major source of contamination to the soils.

- Pathogenic microorganisms in soils irrigated with wastewater may extend into lower profiles of the soil. However, factors such as the sieving effect of soil (influenced by soil texture), higher pathogen die-off rates, and reduced soil moisture could have contributed to lower counts of coliforms and helminthes in the lower profiles of soil.

6.2 RECOMMENDATIONS

- To protect the health of the population it is not enough merely to check the sanitary quality of water used for human consumption, using laboratory analyses in order to obtain information such as the concentration of a certain pathogenic micro-organism or, to establish its presence or absence in the samples (Razzolini and Nardocci, 2006). Assessment of the environmental and health risk is a very vital exercise to be performed. Further research studies should be carried out to assess the environmental and health risk posed by such contamination levels observed in the soils.

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APPENDICES

APPENDIX 1: ANOVA and LSD analysis for soil pH from different treatment fields

ANOVA FOR 0 – 30 cm

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.309	2	.155	2.120	.133
Within Groups	3.062	42	.073		
Total	3.371	44			

Multiple Comparisons for 0 – 30 cm

pH

LSD

(I) Plots	(J) Plots	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Potable water Irrigated plots	Wastewater Irrigated plots	-.12333	.09859	.218	-.3223	.0756
	Control plots	.07800	.09859	.433	-.1210	.2770
Wastewater Irrigated plots	Potable water Irrigated plots	.12333	.09859	.218	-.0756	.3223
	Control plots	.20133*	.09859	.047	.0024	.4003
Control plots	Potable water Irrigated plots	-.07800	.09859	.433	-.2770	.1210
	Wastewater Irrigated plots	-.20133*	.09859	.047	-.4003	-.0024

*. The mean difference is significant at the 0.05 level.

APPENDIX 1: ANOVA and LSD analysis for soil pH from different treatment fields

ANOVA for 30 – 45 cm

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.262	2	.131	1.823	.174
Within Groups	3.020	42	.072		
Total	3.282	44			

Multiple Comparisons for 30 – 45 cm

pH

LSD

(I) Plots	(J) Plots	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Potable water Irrigated plots	Wastewater Irrigated plots	-.06000	.09792	.543	-.2576	.1376
	Control plots	.12333	.09792	.215	-.0743	.3209
Wastewater Irrigated plots	Potable water Irrigated plots	.06000	.09792	.543	-.1376	.2576
	Control plots	.18333	.09792	.068	-.0143	.3809
Control plots	Potable water Irrigated plots	-.12333	.09792	.215	-.3209	.0743
	Wastewater Irrigated plots	-.18333	.09792	.068	-.3809	.0143

APPENDIX 2A : ANOVA AND LSD ANALYSIS FOR SOIL MOISTURE FROM DIFFERENT IRRIGATED FIELDS AND DIFFERENT SOIL PROFILES

ANOVA FOR 0 – 30 cm

Soil Moisture

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.294	2	2.647	80.635	.000
Within Groups	1.379	42	.033		
Total	6.672	44			

Multiple Comparisons for 0 – 30 cm

Soil Moisture

LSD

(I) Plots	(J) Plots	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Potable water Irrigated plots	Wastewater Irrigated plots	-.20000*	.06616	.004	-.3335	-.0665
	Control plots	.60667*	.06616	.000	.4732	.7402
Wastewater Irrigated plots	Potable water Irrigated plots	.20000*	.06616	.004	.0665	.3335
	Control plots	.80667*	.06616	.000	.6732	.9402
Control plots	Potable water Irrigated plots	-.60667*	.06616	.000	-.7402	-.4732
	Wastewater Irrigated plots	-.80667*	.06616	.000	-.9402	-.6732

*. The mean difference is significant at the 0.05 level.

APPENDIX 2A : ANOVA AND LSD ANALYSIS FOR SOIL MOISTURE FROM DIFFERENT IRRIGATED FIELDS AND DIFFERENT SOIL PROFILES

ANOVA FOR 30 – 45 cm

Soil Moisture

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.457	2	2.729	165.932	.000
Within Groups	.691	42	.016		
Total	6.148	44			

Multiple Comparisons for 30 45 cm

Soil Moisture

LSD

(I) Plots	(J) Plots	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Potable water Irrigated plots	Wastewater Irrigated plots	-.51333*	.04683	.000	-.6078	-.4188
	Control plots	.33333*	.04683	.000	.2388	.4278
Wastewater Irrigated plots	Potable water Irrigated plots	.51333*	.04683	.000	.4188	.6078
	Control plots	.84667*	.04683	.000	.7522	.9412
Control plots	Potable water Irrigated plots	-.33333*	.04683	.000	-.4278	-.2388
	Wastewater Irrigated plots	-.84667*	.04683	.000	-.9412	-.7522

*. The mean difference is significant at the 0.05 level.

APPENDIX 3A: ANOVA AND LSD ANALYSIS FOR MICROBIAL POPULATION FROM TOP SOIL PROFILE (0 – 30 cm) FROM DIFFERENT IRRIGATION TREATMENTS

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Coliforms	Between Groups	86.432	2	43.216	70.667	.000
	Within Groups	25.685	42	.612		
	Total	112.118	44			
Faecal Coliforms	Between Groups	82.345	2	41.173	78.356	.000
	Within Groups	22.069	42	.525		
	Total	104.415	44			
Helminths	Between Groups	2187.911	2	1093.956	35.489	.000
	Within Groups	1294.667	42	30.825		
	Total	3482.578	44			

APPENDIX 3A: ANOVA AND LSD ANALYSIS FOR MICROBIAL POPULATION FROM TOP SOIL PROFILE (0 – 30 cm) FROM DIFFERENT IRRIGATION TREATMENTS

Multiple Comparisons

LSD

Dependent Variable	(I) Plots	(J) Plots	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Total Coliforms	Potable water Irrigated plots	Wastewater Irrigated plots	-.46133	.28555	.114	-1.0376	.1149
		Control plots	2.68200*	.28555	.000	2.1057	3.2583
	Wastewater Irrigated plots	Potable water Irrigated plots	.46133	.28555	.114	-.1149	1.0376
		Control plots	3.14333*	.28555	.000	2.5671	3.7196
	Control plots	Potable water Irrigated plots	-2.68200*	.28555	.000	-3.2583	-2.1057
		Wastewater Irrigated plots	-3.14333*	.28555	.000	-3.7196	-2.5671
Faecal Coliforms	Potable water Irrigated plots	Wastewater Irrigated plots	-.27533	.26469	.304	-.8095	.2588
		Control plots	2.72200*	.26469	.000	2.1878	3.2562
	Wastewater Irrigated plots	Potable water Irrigated plots	.27533	.26469	.304	-.2588	.8095
		Control plots	2.99733*	.26469	.000	2.4632	3.5315
	Control plots	Potable water Irrigated plots	-2.72200*	.26469	.000	-3.2562	-2.1878
		Wastewater Irrigated plots	-2.99733*	.26469	.000	-3.5315	-2.4632
Helminths	Potable water Irrigated plots	Wastewater Irrigated plots	-5.73333*	2.02733	.007	-9.8246	-1.6420
		Control plots	11.06667*	2.02733	.000	6.9754	15.1580
	Wastewater Irrigated plots	Potable water Irrigated plots	5.73333*	2.02733	.007	1.6420	9.8246
		Control plots	16.80000*	2.02733	.000	12.7087	20.8913
	Control plots	Potable water Irrigated plots	-11.06667*	2.02733	.000	-15.1580	-6.9754
		Wastewater Irrigated plots	-16.80000*	2.02733	.000	-20.8913	-12.7087

*. The mean difference is significant at the 0.05 level.

APPENDIX 4A: ANOVA AND LSD ANALYSIS FOR MICROBIAL POPULATION FROM DEEPER SOIL PROFILE (30 – 45 cm) FROM DIFFERENT IRRIGATION TREATMENTS

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Coliforms	Between Groups	67.530	2	33.765	66.517	.000
	Within Groups	21.320	42	.508		
	Total	88.850	44			
Faecal Coliforms	Between Groups	60.585	2	30.292	75.427	.000
	Within Groups	16.868	42	.402		
	Total	77.452	44			
Helminths	Between Groups	164.800	2	82.400	10.424	.000
	Within Groups	332.000	42	7.905		
	Total	496.800	44			

Multiple Comparisons : LSD

Dependent Variable	(I) Plots	(J) Plots	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Total Coliforms	Potable water Irrigated plots	Wastewater Irrigated plots	-.43133	.26016	.105	-.9564	.0937
		Control plots	2.35600*	.26016	.000	1.8310	2.8810
	Wastewater Irrigated plots	Potable water Irrigated plots	.43133	.26016	.105	-.0937	.9564
		Control plots	2.78733*	.26016	.000	2.2623	3.3124
	Control plots	Potable water Irrigated plots	-2.35600*	.26016	.000	-2.8810	-1.8310
		Wastewater Irrigated plots	-2.78733*	.26016	.000	-3.3124	-2.2623
Faecal Coliforms	Potable water Irrigated plots	Wastewater Irrigated plots	-.60667*	.23141	.012	-1.0737	-.1397
		Control plots	2.10133*	.23141	.000	1.6343	2.5683
	Wastewater Irrigated plots	Potable water Irrigated plots	.60667*	.23141	.012	.1397	1.0737
		Control plots	2.70800*	.23141	.000	2.2410	3.1750
	Control plots	Potable water Irrigated plots	-2.10133*	.23141	.000	-2.5683	-1.6343
		Wastewater Irrigated plots	-2.70800*	.23141	.000	-3.1750	-2.2410
Helminths	Potable water Irrigated plots	Wastewater Irrigated plots	-3.60000*	1.02663	.001	-5.6718	-1.5282
		Control plots	.80000	1.02663	.440	-1.2718	2.8718
	Wastewater Irrigated plots	Potable water Irrigated plots	3.60000*	1.02663	.001	1.5282	5.6718
		Control plots	4.40000*	1.02663	.000	2.3282	6.4718
	Control plots	Potable water Irrigated plots	-.80000	1.02663	.440	-2.8718	1.2718
		Wastewater Irrigated plots	-4.40000*	1.02663	.000	-6.4718	-2.3282

*. The mean difference is significant at the 0.05 level.

APPENDIX 5A: ANOVA AND LSD ANALYSIS FOR HELMINTH EGG POPULATION AT BOTH DEPTHS FROM DIFFERENT IRRIGATION TREATMENTS

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Upper Profile	Between Groups	2187.911	2	1093.956	35.489	.000
	Within Groups	1294.667	42	30.825		
	Total	3482.578	44			
Lower Profile	Between Groups	164.800	2	82.400	10.424	.000
	Within Groups	332.000	42	7.905		
	Total	496.800	44			

Multiple Comparisons

LSD

Dependent Variable	(I) trt	(J) trt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Upper Profile	potable water irrigated plots	wastewater irrigated plots	-5.73333*	2.02733	.007	-9.8246	-1.6420
		non-irrigated plots	11.06667*	2.02733	.000	6.9754	15.1580
	wastewater irrigated plots	potable water irrigated plots	5.73333*	2.02733	.007	1.6420	9.8246
		non-irrigated plots	16.80000*	2.02733	.000	12.7087	20.8913
	non-irrigated plots	potable water irrigated plots	-11.06667*	2.02733	.000	-15.1580	-6.9754
		wastewater irrigated plots	-16.80000*	2.02733	.000	-20.8913	-12.7087
Lower Profile	potable water irrigated plots	wastewater irrigated plots	-3.60000*	1.02663	.001	-5.6718	-1.5282
		non-irrigated plots	.80000	1.02663	.440	-1.2718	2.8718
	wastewater irrigated plots	potable water irrigated plots	3.60000*	1.02663	.001	1.5282	5.6718
		non-irrigated plots	4.40000*	1.02663	.000	2.3282	6.4718
	non-irrigated plots	potable water irrigated plots	-.80000	1.02663	.440	-2.8718	1.2718
		wastewater irrigated plots	-4.40000*	1.02663	.000	-6.4718	-2.3282

*. The mean difference is significant at the 0.05 level.

APPENDIX 6A: ANOVA AND LSD ANALYSIS FOR IRRIGATION WATER USED ON VEGETABLE FARMS

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Coliform	Between Groups	426.034	2	213.017	298.256	.000
	Within Groups	29.997	42	.714		
	Total	456.031	44			
Faecal Coliform	Between Groups	243.743	2	121.871	241.557	.000
	Within Groups	21.190	42	.505		
	Total	264.933	44			
Helminth	Between Groups	1726.978	2	863.489	105.631	.000
	Within Groups	343.333	42	8.175		
	Total	2070.311	44			

Multiple Comparisons

Dependent Variable	(I) Irrigation water source	(J) Irrigation water source	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Total Coliform	shallow well	stream	-1.54398*	.30859	.000	-2.1667	-.9212
		piped water	5.61671*	.30859	.000	4.9940	6.2395
	stream	shallow well	1.54398*	.30859	.000	.9212	2.1667
		piped water	7.16069*	.30859	.000	6.5379	7.7835
	piped water	shallow well	-5.61671*	.30859	.000	-6.2395	-4.9940
		stream	-7.16069*	.30859	.000	-7.7835	-6.5379
Faecal Coliform	shallow well	stream	-1.33153*	.25936	.000	-1.8550	-.8081
		piped water	4.13471*	.25936	.000	3.6113	4.6581
	stream	shallow well	1.33153*	.25936	.000	.8081	1.8550
		piped water	5.46624*	.25936	.000	4.9428	5.9897
	piped water	shallow well	-4.13471*	.25936	.000	-4.6581	-3.6113
		stream	-5.46624*	.25936	.000	-5.9897	-4.9428
Helminth	shallow well	stream	-8.53333*	1.04401	.000	-10.6402	-6.4264
		piped water	6.60000*	1.04401	.000	4.4931	8.7069
	stream	shallow well	8.53333*	1.04401	.000	6.4264	10.6402
		piped water	15.13333*	1.04401	.000	13.0264	17.2402
	piped water	shallow well	-6.60000*	1.04401	.000	-8.7069	-4.4931
		stream	-15.13333*	1.04401	.000	-17.2402	-13.0264

*. The mean difference is significant at the 0.05 level.