KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

Microbial Quality of Poultry Manure and Selected Vegetables from the Korle-Bu Vegetable Farms

By
Gloria Korantemaa Odame-Asiedu

A Thesis submitted to the Department of Food Science and Technology,

Faculty of Biosciences,

College of Science,

In Partial Fulfillment of the Requirements for the Award of Master of Science Degree in

FOOD QUALITY MANAGEMENT

May, 2018

DECLARATION

I hereby declare that this submission is my own work towards the MSc. degree in Food Quality Management degree 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.

………………………………. ..…..…………………………………..

Date Gloria Korantemaa Odame-Asiedu (PG 8140312

………………………………. …………………………………….

Date Dr. F. C. Mills-Robertson

Supervisor

Certified by

………………………………. ……………..………………………

Date Dr (Mrs) F. Wireko-Manu

Head of Department

ACKNOWLEDGEMENT

My heartfelt praise to God; without Him I can do nothing. I am also sincerely grateful to Dr. Felix Mills Robertson who supervised this work. Thank you, sir, this work would not have been completed successfully. Mr. Isaac W. Ofosu, I owe you my gratitude for encouraging me when I almost gave up. Endless thanks to my husband, Mr. Nicholas Odame-Asiedu, for all the support. I could never get another you. I am grateful to my parents, Mr. John Asare Nyankumah and Ms. Ellen Avorgbedor, for their love and support all these years. To all my friends and loved ones especially Mawusi and Clara, God remember you for good. To Aunty Charlotte, of the Institute of Distance Learning office, God richly bless you. God bless you all.

ABSTRACT

Many vegetable growers in Ghana use animal manure to improve soil quality for vegetable cultivation. Manure use on vegetable farms has been implicated in most intestinal parasitic infection of vegetable consumers worldwide. This study investigated the microbial quality of poultry manure and selected vegetables from the Korle-Bu Vegetable Farms. Thirty-three (33) farmers out of the reported 76 farmers were interviewed employing a face to face interview using a structured questionnaire to ascertain manure use and management practices. All farmers interviewed were males, with majority between the ages of 21 and 40 years (54.54%) with no formal education (69.70 %). Convenient sampling was done from three farms where manure and vegetables samples were collected and tested for microbial quality. Most (85%) cultivated exotic vegetables and employed organic fertilizer (78%), mainly composted (53.85%). Manure was usually applied after transplanting of seedlings (80.71%) around the crops (50%). Laboratory analysis revealed coliforms ranged from 3.5 x 10^4 to 2.3 x 10^5cfu/g for manure samples, 1.3 x 10^3 to 2.0 x 10^3cfu/g for lettuce, 2.2 x 10^2 to 2.1 x 10^3cfu/g for carrots and 1.7 x 10^2 to 1.5 x 10^3cfu/g for green pepper. The microbial load on vegetables were within the WHO acceptable limit on the vegetables and such information can be disseminated to policy makers and consumers to encourage consumers to patronize these organic produces. *Escherichia coli*, and *Salmonella* spp. were observed in manure samples while *Escherichia coli* was observed in one lettuce sample. Farmers need further education on manure handling and use.

TABLE OF CONTENTS

LIST OF PLATES

LIST OF TABLES

TABLE **PAGE**

CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND INFORMATION

Fresh vegetables are progressively becoming food substance for local and export markets. According to Slavin and Loyd, (2012) vegetables are a boundless store that improves the sustenance and well-being of consumers because they are great sources of vitamins, minerals and proteins needed for the suitable functioning and growth of the human body.

Frequent intake of fresh vegetables and fruits have been linked with many health benefits and therefore encouraged by global nutrition and well-being institutions. Vitamins, minerals and high-water content of fruits and vegetables contribute to improving the quality of foods made from them. They are good sources of vitamin C, carotene, thiamine/vitamin B12, niacin and riboflavin. They are also rich in minerals like iron as well as phyto-nutrients (non-nutritive elements which contribute to prevention of degenerative diseases). Phyto-nutrients work as antioxidants to mop up free radicals before they can damage DNA cell membrane and fat containing molecules such as cholesterol. A high consumption of fresh vegetables and fruits in the diet is positively linked with the prevention of heart diseases, diabetes and osteoporosis (Van Duyn and Pivonka, 2000; Slavin and Loyd, 2012). The World Health Organization (WHO) advocates a daily intake of 400 grams of fruits and vegetables for good health and nutrition (WHO/FAO, 2003).

According to the Korle-Bu vegetable farmers, vegetables like lettuce, carrot, cabbage, cucumber, sweet pepper and spring onion are generally not ingredients in the traditional Ghanaian diet; however, their demand has increased among urban dwellers from street food vendors and in canteens and restaurants who regularly buy from them. In Accra, the capital city of Ghana, for example, there are about 800-1,000 farmers engaged in commercial urban vegetable farming where the vegetables grown are eaten by more than 200,000 urban dwellers day-to-day (Obuobie *et al*., 2006). In Kumasi and its surroundings, more than 12,000 farmers are involved in vegetable farming during the dry seasons (Cornish *et al.* 2001). Also urban farmers do cultivate about 90% of the vegetables eaten in the city. Vegetable cultivation contributes significantly to the socio-economic development by creating employment for a section of the population as well as ensuring food security, provision of raw materials for local industries, and generation of foreign exchange (Nouhoheflin *et al.*, 2004; Obuobie *et al*., 2006).

Animal manure application for improving soil fertility in vegetable cultivation is a common practice among vegetable farmers in Ghana. A preliminary field survey conducted by this study author, of vegetable growers at the Korle-Bu farms, indicated that most farmers use animal manure in fertilizing the soil and only switch to NPK fertilizers when the animal manure is in short supply. According to Amoah *et al.* (2007), about 70% of vegetable producers in Accra and Kumasi use poultry manure with only 37% and 5% using NPK fertilizer (mostly at the nursery stage) in Accra and Kumasi, respectively. Animal manure is a cheaper alternative to inorganic fertilizers as many vegetable farmers are peasant farmers, with little financial capital for farming. Although this practice proves beneficial in the wake of several calls for consumption of organic foods, there are several associated challenges, including the possible transfer of pathogenic microorganisms to vegetables from manure.

1.2. PROBLEM STATEMENT

The use of uncured animal manure on vegetable farms has been implicated in the increasing transmission of intestinal parasitic infection to vegetable consumers on the African continent. Some studies on vegetables farms in Ghana have been shown to be associated with a high microbial infection risk (Mensah *et al*., 2001; Obeng-Ofori *et al.*, 2007 Donkor *et al.*, 2010; Golly *et al*., 2016). Although studies on the microbial contamination of vegetables have been reported in Ghana, little is known about the microbial quality of organic manure used in the cultivation of vegetable in the Accra Metropolis.

The study, therefore, sought to assess the microbial quality of organic manure and selected vegetables being used for the cultivation of these vegetables at the Korle-Bu Vegetable Farms.

1.3. JUSTIFICATION

Farmers' knowledge on manure management and the potential risk of animal manure in vegetable production and the presence of microorganisms' need to be evaluated for effective education and training of farmers and consumers.

This study is expected to provide data on the presence or absence of some pathogenic microbes on vegetables grown with animal manure at the Korle-Bu Vegetable Farms, as well as information on the levels of indicator microorganisms on them.

1.4. MAIN OBJECTIVE

To assess the microbial quality of poultry manure and selected vegetables from the Korle-Bu vegetable farms

1.5. SPECIFIC OBJECTIVES

- To assess farmers' knowledge on good manure management practices
- To determine the microbial load of poultry manure and selected vegetables from the Korle-Bu Vegetable Farms
- To determine the types of microbe on the poultry manure and selected vegetables from the Korle-Bu Vegetable Farms

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. URBAN AND PERI-URBAN AGRICULTURE

Migration of people to urban centers in search of better lives has caused the upsurge in urban and peri-urban populace growth, consequently increasing demand for food in these areas. Urban and peri-urban farming is necessary to provide the nutrition necessity of urban dwellers and involves the production, processing and distribution of agricultural produce (plant and animal products as well as ornamentals and flowers) within and around urban areas (Mougeot, 2000). According to the United Nations Development Programme (UNDP), about 800 million persons are directly involved in urban and peri-urban agriculture worldwide (Smit *et al.*, 1996). Of these, 200 million are market producers, employing 150 million people full-time who produce easily perishable crops like vegetables for distribution and sale in markets.

Currently, urban and peri-urban vegetable cultivation caters for almost all perishable vegetables consumed by residents in Ghana's cities. Generally grown organic vegetables in Ghana are lettuce, cabbage, green pepper, carrot, tomato, garden eggs, green beans and spring onions, which are often used in exotic diets and frequently eaten raw. Kumasi has about 41 ha being used for urban vegetable farming and 12,000 ha for peri-urban vegetables farming, especially, in the dry season, with most of the land belonging to government institutions or private developers (Cornish and Lawrence, 2001). The major vegetable farming sites in Kumasi include; Gyinyaase (also known as Engineering which is the largest vegetable farming site in Kumasi of about 21.8 ha), Georgia Hotel (0.4 ha) and D-Line (also known as Weweso with about 3.1 ha of cultivated land). In Tamale, about 40 % of the vegetable farmers cultivate their land year-round, with 52% depending on polluted water sources (Zibrilla and Salifu, 2004). The major vegetable farming sites in Tamale include; Builpela/Bupeila (2.6 ha), Sangani (0.5 - 4 ha), Water Works (13.5 - 22 ha), and Zagyuri (7 - 12 ha) (Obuobi *et al.*, 2006).

In Accra, about 60% of the 800-1000 vegetable farmers produce exotic vegetables and with about 40% producing traditional vegetables. There are five major vegetable production sites in the Accra Metropolis, comprising Korle-Bu, Dzorwulu, La Marine Drive, GBC and CSIR farming sites. At the Korle-Bu farms, vegetables are cultivated on land belonging to the Korle-Bu Teaching Hospital and the land is cultivated by junior hospital staff who are mostly indigenes from the northern regions of Ghana as well as Burkina Faso with the aim to supplement their income. It is reported that out of about 76 farmers who cultivate the Korle-Bu Farms, only one is a female. The water used to irrigate the farms is derived from drains, which pass through the hospital compound and staff flats (Obuobi *et al.*, 2006).

2.2. THE USE OF MANURE IN AGRICULTURE

Before the advent of synthetic fertilizers, animal manure was used to enrich the soil for the cultivation of crops. The difficulties associated with transporting and application of animal manures due to their bulkiness, as well as the associated risk of contamination of crops with harmful microorganisms advance the shift from the use of animal manures to synthetic fertilizers on farms. Another reason for the shift from animal to synthetic fertilizers is the recent unavailability and its high demand for growing organic foods. In recent years, synthetic (inorganic) fertilizers and other chemicals employed in agriculture have generated several food safety and environmental quality concerns worldwide. Most health-conscious consumers now call for organic foods, revamping the use of animal manures and other organic soil enhancement techniques in farming (Worthington, 1998; Worthington 2001; Masarirambi *et al*., 2010).

Organic farming is a farming system which employs organic manure, while avoiding or reducing to the barest minimum, the application of synthetic fertilizers, pesticides and chemicals (Gil *et al.*, 2000). The main differences with organic farming and conservative farming practices arise from soil and pest management aspects of farming (Philips and Peterson 2001). Organic farming holds promise in avoidance of the contamination of soil and water bodies with chemical residue and runoffs. This has caused the speedy emergence of the organic food as a significant food industry in the U.S.A and other countries since the early 1980s (Lohr 1998; Thompson, 1998).

Organic farming system relies on environmentally friendly practices such as biological pest management and composting; excluding the use of synthetic chemicals and antibiotics all together (Natvig *et.al*, 2002). Due to the high cost of inorganic fertilizers and pesticides as well as the inability of synthetic fertilizers to condition the soil, organic farming system presents a more profitable, environmentally sustainable and energy efficient farming system than conventional farming systems. However, yield of produce from organic farms is similar to that of inorganic farms. Organic products have also been found to taste better than 'non-organic' foods (Reganold *et al.*, 2001).

Although organic farming is yet to gain popularity among developing countries in sub-Saharan Africa, most farmers involved in the production of vegetables in Ghana apply animal manure and hardly use chemical fertilizers on their farms. A survey on the use of organic manure among vegetable farmers in the Shama, Birim and Sissala districts of Ghana indicated that most farmers (75% of respondents) applied fertilizers to their vegetable farms with organic fertilizers used predominantly (about 71.11% of fertilizer users). Most farmers (89.53%), who did not apply any form of fertilizer to their farms, expressed interest to use organic manure on their farms rather than inorganic fertilizers. Bulkiness and difficulty of transporting organic manure from source to farm was the main limiting factor to its use. Education of farmers on several aspects of organic manure use (processing of organic manure, timing of application, placement of the manure, and the proper amount of manure to be used) will be necessary to improve the efficient use of organic manure by farmers (Agyarko and Adomako, 2007). Most urban vegetable growers in the Accra and Kumasi Metropolis of Ghana only revert to inorganic fertilizers like NPK when the animal manure is in short supply or use them at the nursery stage (Amoah *et al*, 2007).

Research works done to ascertain ‘the effects of various levels of farmyard manure and calcium ammonium nitrate (CAN) on vegetative growth, produce and quality (Vitamins A and C, Nitrates) of *Solanum villosum* in Keiyo district of Kenya’ showed that harvest was increased for crops grown with organic manure (Kikposgei *et al*., 2003). They observed that organic manures at high levels (20t/ha) increased, while application of CAN at 200-400 kg/ha decreased vitamin C content in both young and older tissues. For the interactive effect of variety and fertilizer, the interaction of Atlas 70 cabbage variety and poultry manure gave the highest weight of whole plant, gross yield, marketable yield and economic production (Hassan and Solaiman, 2012).

2.2.1. Forms of Animal Manure Used in Agriculture

Depending on the solid or dry matter content, manures are generally classified into liquid, slurries or solids.

2.2.1.1. Liquid Manure

Liquid manure contains between 0 – 5% dry matter. They contain lower nutrients concentrations than solid or slurry manures. This implies that liquid animal manure may be applied at relatively high volumes. However, the general recommendation for their slow rate of application includes, not to be applied at rates that exceed the soil infiltration rate, nor exceed the amount needed to bring the soil to field water holding capacity (Johnson and Eckert, 1995; Hoorman *et al*., 2008).

2.2.1.2. Slurry or Semi-Liquid Manure

Manures with about 5 – 15% dry matter content are termed slurries. Slurries can be applied onto farms by slurry tankers which spray the slurries under high pressure. Slurries tend to be more contaminated with microorganisms than solid. Slurries have high moisture content as well as available carbon, which favours the proliferation of pathogens. When slurries are stored over a period of time without addition of fresh slurry, their pathogen loads decrease. This probably is because nutrients are used up and more by-products of metabolism are present in the slurries which make them unfavourable for microbial growth. A study found *Salmonella*, *Listeria monocytogenes*, and *E. coli* to be more prevalent in manure slurries than in solid manures. (Sobsey *et al*.,2006)

2.2.1.3. Solid Manure

Solid manures contain more than 15% dry matter content. Although solid manures cannot be sprayed onto farms, manure which contains about 15 – 20% dry matter could be pumped using heavy duty pumping equipment onto farms. Solid manures present more ease of storage than liquid manures. They could be composted or dried into powder. Solid manures could include the beddings, feathers and feed wastes from the farms. Such manure is termed litter manure. (Sobsey et al, 2006)

Types of Manure

- Poultry manure

- Ruminant manure
- Swine manure

2.2.2. Poultry Manure

Poultry manure is reported to be the most valuable source of plant nutrients. It is richer in nitrogen compared to other animal manures. Also, broiler litter dries under normal house conditions and will average about 20 percent moisture, which concentrates the nutrients in poultry manure (Chen and Jiang, 2014).

In Ghana, the type of manure used on vegetable farms depends on its availability. According to Lopez-real (1995), large quantities of poultry manure are generated by poultry farms in the peri-urban areas of southern Ghana.

There are more than 300 poultry farms in and around Kumasi with a very varied array of bird size. The Veterinary Services Directorate (VSD) in Ghana recorded 3.7 and 1.9 million poultry in the Greater Accra and Ashanti Region of Ghana, respectively, in the year, 1994. (Lopez-real 1995) This implies that poultry manure is readily available and mostly used for urban and peri-urban agriculture in Ghana.

Shiyam and Binang (2013), investigated ‘the effect of poultry manure and plant population on productivity of fluted pumpkin (*Telfaiaria occidentalis* Hook F.) in Calabar, Nigeria’ and found that longest vines, most leaves per plant, highest fresh leaf weight and correspondingly highest dry matter yield were obtained by applying poultry manure at 24 t ha^{-1} to 20,000 plants ha^{-1}. This indicates that the application of poultry manure at 24 t ha^{-1} and plant population density of 20,000 plants ha^{-1} will give optimum yield of fluted pumpkins in the Calabar area of Cross River State, Nigeria’ and can boost fluted pumpkin production and help improve livelihood of farmers.

2.3. NUTRIENT COMPOSITION AND AVAILABILITY OF MANURE

The nutritional composition of manure varies considerably, depending mainly on the animal species and the composition of the diet fed them. Poultry manure (no litter) is reported to have higher amounts of Nitrogen than other animal manures as shown in Table 2.1. Other factors include; animal genetics, animal performance, production management, facility type, bedding, as well as manure collection, storage, handling and agitation for land application (Chen and Jiang, 2014).

Most microorganisms present in the digestive tracts of livestock are excreted with the manure; their continuous metabolism results in the release of gaseous compounds like ammonia and the depletion of nutrients in the manure with time.

Table 2.1: Nutritional Composition of Different Animal manure

Source	% moisture	N%	P_2O_5%	K_2O%
Beef cattle feedlot	68	0.7	0.6	0.9
Dairy cattle	79	0.6	0.2	0.6
Liquid dairy	91	0.2	0.05	0.2
Swine	75	0.5	0.3	0.5
Liquid swine	97	0.1	0.1	0.1
Horse	70	0.7	0.2	0.7
Sheep	65	1.4	0.5	1.2
Poultry (no litter)	54	1.6	0.9	0.4
Liquid poultry	92	0.2	0.04	0.3

(Courtesy: Seefeldt, 2015)

Nutrient availability of manure refers to the amount of nutrients present in manure in a form (chemical or physical) which allows for immediate uptake or utilization by plants. Nutrients in manure, being mostly organic, are not readily released for plant growth. In the soil, nutrients in manure undergo microbial transformations to inorganic forms, making them available for plant uptake. This ‘slow’ release of nutrients from manure implies that the effects of manure application on the soil can endure up to several years. Table 2.2 below shows the nutrient availability for different animal manure sources.

Table 2.2: First-Year Nutrient Availability for Different Animal Manure Sources

SOURCE	NITROGEN %	PHOSPHORUS %	POTASSIUM %
Beef cattle (solid or liquid)	30–50	80–100	90–100
Dairy (solid or liquid)	30–50	80–100	90–100
Liquid swine (anaerobic pit)	90–100	90–100	90–100
Liquid swine (anaerobic lagoon)	90–100	90–1003	90–100
Poultry (all species)	50–60	90–100	90–100

(Courtesy: Sawyer and Mallarino, 2016)

2.4. MICROBIAL QUALITY OF MANURE

Some gut micro-flora is excreted with animal faeces. Although most intestinal microorganisms are not harmful, a widespread variability of pathogenic viruses, bacteria and parasites may be found in the faeces of wild and domestic animals. (Sobsey *et al*., 2006).

Table 2.3 shows bacteria associated with faecal matter from livestock and poultry, including *Escherichia coli (E. coli)* O157:H7 and other Shiga-toxin producing strains, *Salmonella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, *Shigella* spp., *Listeria monocytogenes*, *Leptospira* spp., *Aeromonas hydrophila*, *Clostridium perfringens*, and *Bacillus anthraxis* (in endemic area) in mortality carcasses.

Table 2.3: Occurrence, Infective Doses, and Diseases of Some Pathogens Present in Manure

Pathogen	Probability of Occurrence in Manure (%)			Infective Dose CFU/g	Disease Caused
	Cattle	Poultry	Swine		
Salmonella spp.	0.5-18	0-95	7.2-100	100- 1,000 cells	*Salmonella enteritis*, Typhoid Fever, Paratyphoid fever
E. coli 0157:H7	3.3-28	0	0.1-70	5- 10 cells	Enteric colibacillosis
Campylobacter spp.	5-38	57-69	14-98	<500 cells	*Campylobacter enteritis*
Yersinia enterocolitica	-	-	0-65	10,000,000 cells	Yersiniosis
Listeria spp.	0-100	8	5.9-20	<10,000 cells	Listeriosis

(Courtesy: EPA-OW, 2013)

Parasites such as *Giardia lamblia*, *Cryptosporidium parvum*, *Balantidium coli*, *Toxoplasma* gondii, *Ascaris suum* and *A. lumbricoides*, *Trichuris trichuria* as well as viruses like Rotavirus, hepatitis E virus, influenza A (avian influenza virus), enteroviruses, adenoviruses, calici viruses (norovirus) are associated with faecal matter from livestock and poultry (USEPA 2004, Rogers and Haines 2005; Sobsey *et al*., 2006; Pappas *et al*., 2008; Bowman et al., 2009). Therefore, the application of uncured fresh manure on farms poses high risk of contamination of soil and crops. Exposure of manure to sunlight or heat, such as generated during composting, kills pathogens in manure.

2.5. MICROBIAL QUALITY OF READY-TO-EAT VEGETABLES

Pathogenic microrganisms most commonly linked with vegetables include *Salmonella*, *Shigella*, *E. coli* O157, *Listeria*, *Campylobacter*, *Cryptosporidium* and also viruses such as Hepatitis A. Contamination of vegetables may ascend as an import of treating soil with manure, contaminated irrigation water, as well as poor harvesting, transportation and processing of these vegetables.

Japan recorded the world's biggest outbreak due to vegetables in 1996 when over 11,000 people were infected with *E. coli* O157:H7, resulting in the death of 3 school children. About 6,000 infected persons were culture confirmed for the pathogen ('Ministry of Health and Welfare of Japan', 1997). Vegetable borne outbreaks are, however, low in Europe and USA.

Contamination of lettuce with *Salmonella* spp. is a common source of food poisoning outbreaks associated with fresh produce. Parsley contaminated with shiga-toxin producing *Citrobacter freundii*, was implicated in food poisoning of pupils and staff of a nursery school in Germany (Tschäpe *et al*., 1995). *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Serratia marcescens*, *Serratia rubidaea*, *Enterobacter cloacae*, *Kluyvera ascorbata*, *Pantoea agglomerans*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* were identified in fresh vegetables and salads produced from them (Falomir *et al*., 2010). Microbiological screening of cucumber, cabbage, carrot, and lettuce from a Yaba market in Lagos, Nigeria indicated the presence of *E. coli* O157:H7 on all salad vegetables and *L. monocytogenes* in cabbage and lettuce (Uzeh and Adepoju, 2013). Several investigators have also identified *E. coli*, and *Listeria monocytogenes* in organic vegetables (McMahon and Wilson 2001; Sagoo *et al*., 2001; Loncarevic *et al*., 2005). 'In Ghana, high levels of faecal contamination have been reported in irrigation water and on vegetables grown in cities ranging between 3.00 and 8.30 $\log_{10}$ units per 100-gram faecal coliform concentration' (Amoah *et al*., 2007).

Tiimub *et al*. (2012), investigating 'the bacterial contamination levels of lettuce irrigated with waste water in the Kumasi Metropolis of Ghana' reported bacterial counts higher than the recommended World Health Organization (WHO) and International Commission on Microbiological Specifications for Food (ICMSF) standards of 10^3. The lettuce harbored 4.93×10^4 to 6.17×10^4 CFU total coliforms, 3.48×10^3 to 4.66×10^4 CFU faecal coliforms, 2.98×10^3

to 3.86 × 10^4 CFU *E. coli,* 2.50 × 10^2 CFU to 2.72 × 10^2 CFU *Samonella* and 0.68 × 10^0 to 2.05× 10^0 CFU enterococci. Contamination of the lettuce was attributed to the waste water used in irrigating the farms, although this assertion was not established. However, since it is common practice for farmers to use animal manure on vegetable farms, possibility of manure as the source of contamination cannot be ruled out. It is therefore necessary to establish the actual source of contamination of the vegetables.

2.5.1. Influence of Manure on Microbial Quality of Vegetables

Microorganisms in uncured manure can be transferred into the soil and the crops grown on them. Studies on 'the influence of cattle manure on the bacteriological quality of organic Iceberg lettuce' by Frøseth *et al*. (2005), revealed that though there were differences in the levels of bacteria in the soil fertilized with organic and inorganic fertilizers one week after fertilizer application, the levels of faecal indicator bacteria in the lettuce at harvest were low and there was no significant difference in the bacteriological quality of the lettuce among the different fertilizer treatments.

Three different vegetables (lettuce, radish and spinach) grown with different organic manures and mineral fertilizer (control) were evaluated for microbiological quality. It was found that all the vegetables analyzed had low microbial counts, making them acceptable for consumption (Machado *et al*., 2006.)

Also, there were no significant differences between total and faecal coliform counts on spinach and radishes as well as faecal coliform count in lettuces grown with the different fertilizers. However, differences were observed in the total coliform counts of lettuces grown with the different fertilizers, with bovine and chicken litter yielding higher total coliforms (Machado *et al*., 2006).

‘Pre-harvest evaluation of coliforms, *Escherichia coli, Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers’ indicated that *E. coli* was about 6 times more prevalent in organic produce than conventional produce (Mukherjee *et al*., 2004). Although *E. coli* O157:H7 was not detected in any of the produce analyzed, *Salmonella* was isolated from one organic lettuce and organic pepper sample (Mukherjee *et al*., 2004).

2.6. SURVIVAL OF PATHOGENS IN MANURE, SOIL AND VEGETABLES

Several factors influence the prevalence of microorganisms in crops grown with animal manure. These include; the type of manure, the type of crop, the time of application of organic manure on farms as well as the type and viability of the microorganism.

2.6.1. Type of Manure

According to Li *et al.* (2007), the type of animal (species) exerts a high influence on the pathogen load of their faeces. *E. coli* is frequently present in the faeces of ruminants (cattle, goat, AND sheep among others). The most frequently isolated pathogen in poultry faeces is *Salmonella*. *Campylobacter* is also quite frequently associated with poultry faeces. The incidence of *Salmonella* in poultry faeces can range widely from 0 to 100%, with the population of *Salmonella* in chicken litter ranging from 4 to 1.1×10^5 MPN/g litter. The age of the bird could affect the prevalence of *Salmonella* in its faecal samples. A study found layer birds at 18 weeks old to have the highest *Salmonella* prevalence of 55.6%, followed by 41.7% in 25 to 28 weeks old birds, 16.7% in 75 to 78 weeks old birds and 5.5% in 66 to 74 weeks old birds. (Li *et al* 2007)

2.6.2. Age of Manure

The microbial population in manure usually declines during storage, with extended storage exerting the most impact. This is because with storage and extended storage there is usually competition among the microrganisms and with the heat build-up most microrganisms die Mukherjee *et al*. (2004), reported that organic farms employing manure composts aged a year old or less had incidence of *E. coli* 19 times more than those of farms employing older manure (Table 2.4).

2.6.3. Time of Application of Manure

Manure is usually applied to the soil prior to sowing of seeds or transplanting onto the field. Sometimes, the manure is applied after sowing or transplanting. In the latter case, the risk of manure falling directly on vegetables and introducing contamination to them is high. *E. coli* was found to persist more in soils fertilized with manure in the spring season than in the fall season (Mukherjee *et al*., 2004).

2.6.4. Type of Crop

Root crops like radishes and carrots as well as leafy vegetables such as lettuce, having their edible parts touching the soil, are more prone to microbial contamination from the soil. Gagliardi and Karns (2002), reported that *E. coli* O157:H7 persisted for a longer period of time with cover crops. The same trend was observed by Islam *et al*. (2004), who found that *E. coli* O157:H7 persisted for a longer time in soil covered with parsley plants than in soil from lettuce plots, which were bare after the lettuce plots were harvested. Seedlings display exceptional threat potential, since the sprouting stage disrupts the barrier of the seed, causing pathogens, which may be existing to grow on nutrients from the sprouted plant. Also, unsuitable sanitation during sprouting, following storage and conveyance may result in contamination with pathogenic organisms which may allow favourable environments to be created that will allow certain pathogens to reproduce (Thompson and Powell, 2000).

Table 2.4: *Escherichia coli* Prevalence in Fruits and Vegetables According to Organic Management Practices

Criteria	Group	Total number of Samples	% Positive Samples
Type of Manure	Cattle	157	16
	Other	319	6.6
Age of Manure	12 months or less	95	1.0
	>12 months	223	25.3
Time of Application	Spring	223	5
	Fall	56	10.7

(Courtesy: Mukherjee *et al*., 2004).

2.6.5. Type and Persistence of Microorganism

‘It has been shown that different bacteria have different potential for the colonization of plants’ (Dong *et al*., 2003). *E. coli* O157:H7 was found to be able to persist for a long time in the soil and could pollute crops such as raw vegetables (Khandaghi *et al.*, 2010). *Campylobacter* can survive in moist animal faeces for up to 4 days but can only last between a few minutes to hours in dry conditions and moist heat of about 55 ^{0}C (Collins et.al., 1996; Sobsey *et al*., 2001).

2.7. PATHOGEN DISPERSION IN MANURE, SOIL AND VEGETABLES

The dispersion of pathogens from manure to vegetables could happen via different routes: Application of manure after transplanting of seedlings poses the risk of manure particles as well as microorganisms to drop directly on crops. Water from heavy rainfall or watering equipment could splash soil on vegetables and introduce contaminants from the soil to them. Movement of microorganisms from roots to leaves has also been postulated. (Solomon *et al.*, 2006)

Although Solomon *et al*. (2002), reported 'that *E. coli* O157:H7 was transmitted from the soil to lettuce via plant roots to the lettuce seedling leaves'; when seeds are cultivated in polluted soil results of investigations by Johannessen *et al*. (2004), indicated that no bacterium was transferred from the roots to the lettuce leaves. The conflicting results of the two studies suggest that the time of introduction of contamination could be significant.

2.8. INDICATOR MICROORGANISMS IN MANURE AND VEGETABLES

The presence of certain microorganisms can prove the source of contamination. Such microorganisms are termed indicator microorganisms. *E. coli* and other faecal coliforms are exclusively present in faeces of animals. Thus, the presence of *E. coli* in food is confirmation that contamination is from faecal origin. Other indicator microorganisms include; *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and *Staphylcoccus aureus* among many others. (Guchi *et. al.*,2010)

2.8.1. Escherichia coli

Generally, they are non-disease-causing which form a portion of the regular human and animal gut flora. *E. coli* and other faecal coliforms are common problems when working with untreated manure and have been shown to contaminate crops when proper manure management plans are not implemented. *E. coli* usually contaminates waterways in times of high rain or flooding, but contamination from faulty or leaking manure lagoons or over-application of manure can contaminate waterways and crops during dry weather periods. (Sussman, 1997; Sobsey *et al.*, 2001).

Seven groups of enterovirulent *E. coli* have been identified to be associated with diarrhoea including; attaching and effacing *E. coli* (AEEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAggEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and verocytotoxin-producing *E. coli* (VTEC) (Sussman, 1997; Sobsey *et al*., 2001). Factors for their identification include toxin production, adhesion and invasiveness. Among the serogroups of *E. coli* producing verocytotoxins (VTEC), O157 is the usually identified (Sussman, 1997; Sobsey *et al*., 2001).

2.8.2. *Salmonella* spp.

They cause significant gastric infections in humans. The commonly reported non-typhoidal serotypes are *Salmonella enteritidis* and *Salmonella typhimurium.* They are implicated in outbreaks and have been associated with a diverse range of food borne illneses. Nonetheless, a varied range of serotypes have been linked with epidemics involving fresh yield. Salmonellosis is characterized by watery stool, temperature hikes, abdominal cramps and vomiting typically with a duration of 4-7 days (Hedberg *et al*., 1999; Sobsey *et al*., 2001).

2.8.3. Campylobacter spp.

Species of *Campylobacter* and specifically, *Campylobacter jejuni,* are believed to be the most common microbial cause of gastric illness in humans (Allos et al.,1998). Animals and birds are the chief store of the human pathogenic *Campylobacter* though water is a probable cause for contamination with these organisms (Buswell *et al*., 1999; Mason *et al*., 1999). There is also likelihood for cross-contamination of fresh yield with *Campylobacter* from meat and poultry during food preparation (Beuchat, 1995).

2.9. ENVIRONMENTAL AND HEALTH RISKS ASSOCIATED WITH MANURE FARMING

The use of animal manure presents several environmental and health issues. The stench from animal manures could pollute the environment and cause breathing problems to farmers and residents near farms. This problem is more common in urban and peri-urban farms where farms are situated near residential areas. Human exposure to manure dust has been linked with various infectious, allergic, respiratory and immunologic diseases, with avian influenza from poultry manure dust being of most concern. Other issues arising from application of animal manure include; antimicrobial resistance of pathogenic organisms in animal manure and antibiotic absorbance by plants. (Kumar *et al*., 2004).

2.9.1. Risk of Contracting Avian Influenza Virus

The use of poultry manure poses the risk of farmers contracting avian influenza. Humans can contract the avian influenza virus through direct contact with bird faeces and respiratory secretions, droplets and by transfer through contacts with contaminated formites. The risk is heightened among farmers in developing countries who usually apply poultry manure to farms without protective clothing such as gloves and nose masks (Okiki *et al*., 2010).

Questionnaires were administered to 91 vegetable farmers employing poultry manure in the Ojo Town in Lagos State, Nigeria to assess them for physical and psychological ill-health associated with avian influenza and their knowledge about avian influenza. Similar questionnaires were administered to 100 control subjects that were not vegetable workers and were neither occupationally exposed to organic dust. The study revealed that vegetable farm workers reported significantly higher symptoms of physical ill health than control. (Okiki *et al*., 2010). The prevalence of psychological symptoms of avian flu such as anxiety and depression among vegetable farm workers were not significantly different from the control populace. The knowledge of Avian Influenza risk among vegetable workers was very poor. Application of manure without protective clothing as well as bathing with manure contaminated well water were the exposure routes to the avian influenza risk (Okiki *et al*., 2010).

2.9.2. Anti-Microbial Resistance of Microorganisms in Manure

Another serious issue that arises from manure application is the risk of transferring antimicrobial resistant pathogenic microorganisms to humans. The persistent use of antimicrobials in livestock could make certain microorganisms develop resistance to these antimicrobials. (Levy and Marshall, 2004).

Some bacteria may develop resistance to antimicrobials when their deoxyribonucleic acid (DNA) changes through the mutation of existing genetic material. Bacteria may also develop resistance through conjugation (the transfer of genetic material between living bacteria), transformation (obtaining genetic material from the environment), or transduction (the transfer of genetic material between bacteria via a bacteriophage) (Rogers and Haines, 2005).

Antimicrobial resistant strains resulting pathogenic microorganisms if present in the faeces of livestock applied to farms for soil enhancement and plant growth may be transferred to humans. In such an event, when human hosts of these antimicrobial resistant strains of pathogenic microorganisms are treated with the antimicrobials, symptoms of infection still persist, which may lead to abuse of certain medicines (Levy and Marshall, 2004).

Studies by McEwen and Fedorka-Cray (2002) suggest that the use of antimicrobials in livestock and poultry transferred antimicrobial resistance to *Salmonella* and *Enterococcus*. These bacteria may be transferred to humans through the food chain and via contaminated water.

Golly *et al*., (2016), investigated the 'antibiotic resistance of bacteria isolated from cabbage (*Brassica oleracea*), carrot (*Daucus carota*) and lettuce (*Lactuca sativa*) cultivated on animal manure-enriched soils in Kumasi, Ghana' and found high prevalence of antimicrobial resistant bacteria in the vegetables with extensive Multi antibiotic resistance (MAR) bacteria that showed some level of resistance to almost all the antibiotics tested. They implied from their findings that vegetables are potential vehicle for microbial food poisoning as well as a source of infectious diseases that cannot be treated with commonly used antibiotics.

2.9.3. Antibiotics Absorbance by Plants

Possible human health risks linked to the intake of fresh vegetables cultivated in soil fertilized with antibiotic overloaded manures cannot be ruled out. Antibiotics are commonly added to animal feed as supplements to promote growth of food animals. However, absorption of antibiotics in the animal gut is not complete and as a result substantial amounts of antibiotics are excreted in urine and faeces that end up in manure. Antibiotics could be excreted in a form that may be as high as 90% of the parent compound (Philips *et al*., 2004; Kumar *et al*., 2005). Certain antibiotics cause allergic reactions in some individuals and their presence in plant tissues could prove fatal to such individuals.

Several reports have shown the common antibiotics in beef cattle, swine and turkey manure to include; tetracycline, tylosin, sulfamethazine, amprofourlium, monensin, virginiamycin, penicillin and nicarbazine. These antibiotics have been shown to remain stable in manure, ending up in the agricultural fields which they are applied (Boehm, 1996; De Liguoro *et al*., 2003; Kumar *et al*., 2004, 2005).

Maize, and vegetables such as cabbage and green onion grown with animal manure absorbed the antibiotics chlortetracycline into their tissues, but not tylosin. Also, the concentration of antibiotics absorbed by the plants increase with their increasing concentration in the animal manure (Kumar *et al*., 2005).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. STUDY DESIGN

Convenient sampling where by samples were collected by sectioning the whole area into 3 parts. Samples were picked from each part. The samples collected were common to the 3 parts marked as farm A, B and C.

3.2. STUDY SITE

Three farms at the Korle–Bu vegetable farms located within the Accra Metropolis from which manure and vegetables (lettuce, carrot and green pepper) were collected.

3.3. QUESTIONNAIRE ADMINISTRATION

Face to face questionnaire administration was employed to access manure handling techniques from 33 vegetable farmers.

3.4. SAMPLE COLLECTION AND PROCESSING

Samples of poultry manure and vegetables (lettuce, carrots and green pepper) were collected from each of the three farms in Korle-Bu, Accra Metropolis using aseptic methods into sterile zip lock bags and transported to the Noguchi Memorial Institute for Medical Research (NMIMR) laboratory within 2 h in cool chamber (4 or 8°C) for sample processing.

All buffers and media used for sample preparation and culturing were prepared under aseptic conditions following the manufacturer's protocol (Appendix I).

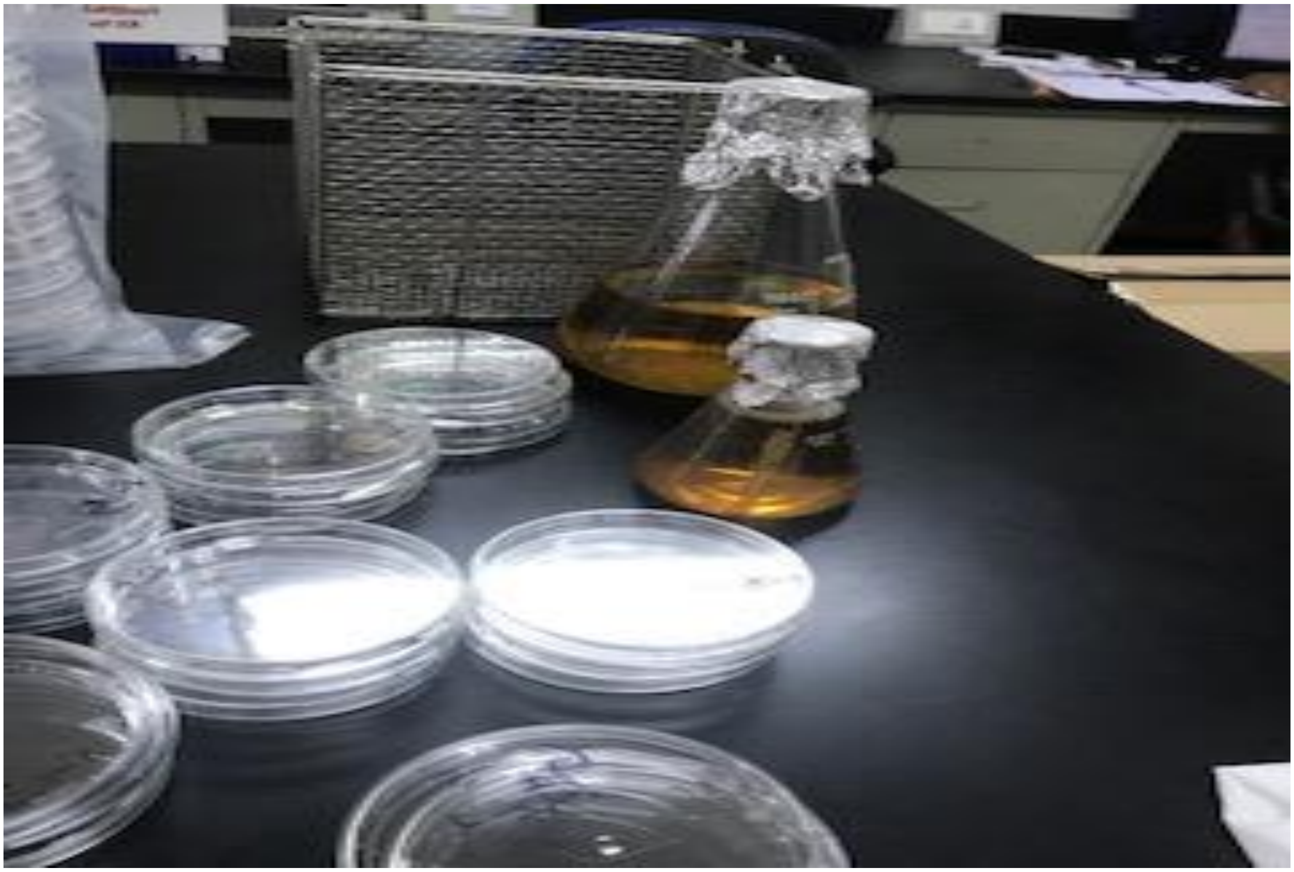


Plate 3.1: Media ready to be poured into petri dishes

Plate count was used to measure the concentration of bacterial cells by determining the number of colonies. It was therefore, used to estimate the number of viable cells in each sample by counting the number of isolated colonies on the nutrient agar.

Each sample was serially diluted in a 10 fold increment as described above. 1ml of each diluted solution was aseptically transferred into a sterile petri dish and then was overlaid with melted nutrient agar that was cooled to about 50°C. The dish was then gently swirled to evenly mix the sample with the liquid agar. When the agar had hardened the plate was then incubated at 37°C for 18-24 h. After incubation, the colonies were counted as Colony Forming Unit (CFU).

The amount of sample diluted and the amount of dilution used in plating was used to calculate/ measure or estimate the concentration of viable cells per milliliter in the original sample. Cells attached to one another were counted as single cells or colony-forming unit (CFU).

3.4.1 Measurement of Bacterial Growth

Viable cell count was used to quantify the number of bacterial cell present in the samples collected.

3.4.1.1. Serial Dilutions

A 90ml aliquot of Phosphate Buffered Saline (PBS) was used to homogenize 10g of each vegetable or manure sample from the three farms at Korle-Bu and shaken in a stomacher blender for 15 min and the supernatant was poured into a sterile test tube and kept as 10^{-1} dilution. Approximately 1ml of the 10^{-1} dilution of each sample was pipetted into 9ml of PBS to make 10^{-2} dilution. This process was applied to the 10^{-2} dilution to obtain the 10^{-3} and subsequently, the 10^{-4} and 10^{-5} dilutions.

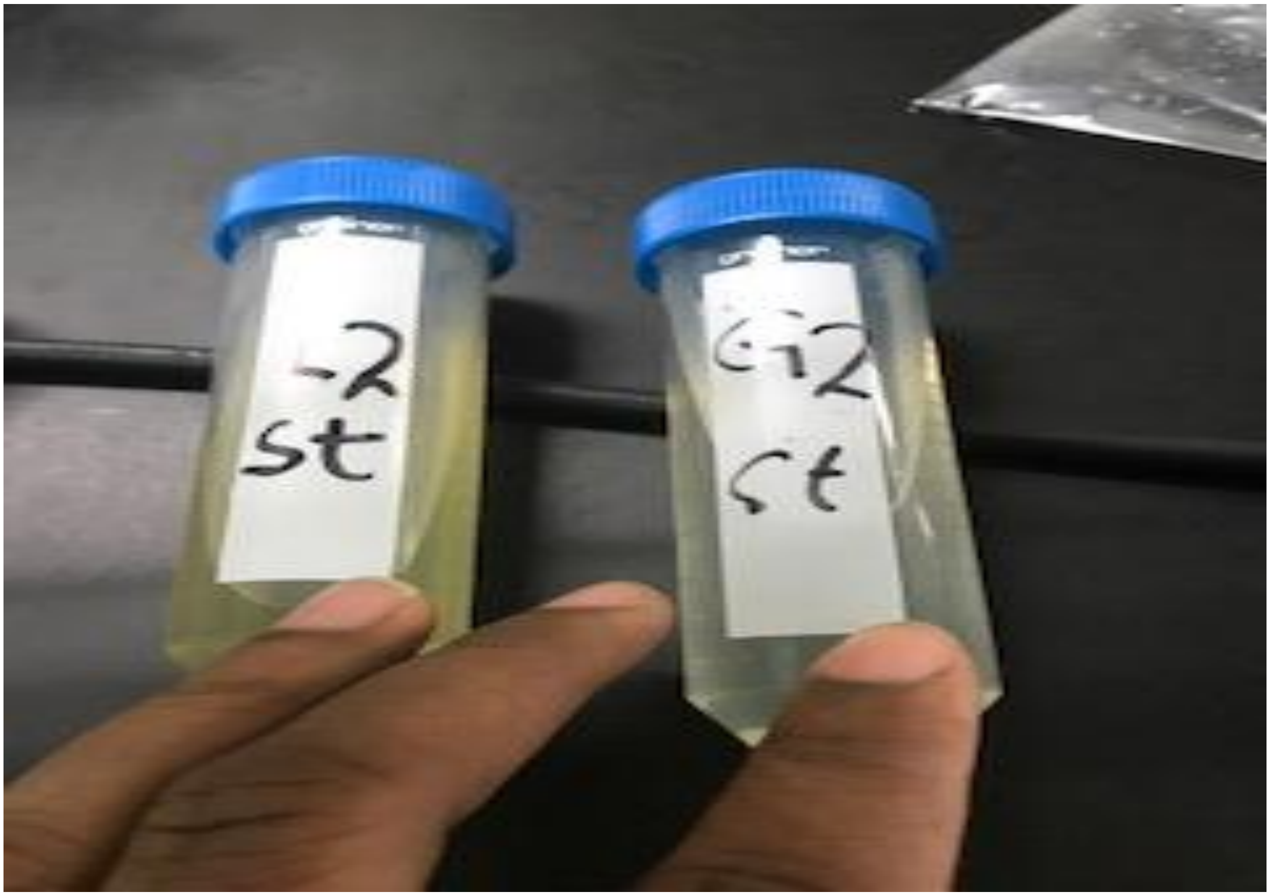


Plate 3.2: Serially diluted samples in tubes

3.4.2. Bacterial Culture (Microbial Detection and Identification)

Sediments from the serial dilution of the various samples were inoculated aseptically under a biosafety cabinet using sterile inoculating loop and streaking on MacConky agar (McA), Blood agar (BA), Salmonella-Shigella agar (SSA) and Mannitol Salt agar (MSA) for detection of *E. coli*, *Bacillus* spp., *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus*, respectively. The inoculated media plates were then incubated overnight at 37°C. After incubation media plates were observed for distinctive colonies.

Culture media with mixed bacterial growths were sub-cultured to obtain a single type of colonial growth. The colonial morphology of each bacterium was then observed for presumptive identification of each isolate. Gram's staining was then performed to observe the microscopy appearance of each isolate. Oxidase test was performed to identify bacteria that produce cytochrome C oxidase. Isolates on selective media McA. SSA and Blood agar were then sub-cultured on Blood agar for identification of bacterial isolates using the 'Analytical Profile Index 20E' (API 20E) from BioMerieux, France.

3.4.2.1. Gram Staining

A drop of 0.85% saline was put on a clean glass slide and with sterile inoculating loop one or two colonies (depending on the size of the colonies) of the fresh bacterial growth was picked and emulsified with the saline and allowed to air dry, then the slide was passed over a flame to heat fix them onto the slide. The surface of the slide was flooded with crystal violet solution, made to stand for about 1 min and then washed under running water. The slide was then flooded with iodine solution, made to stand for 1 min and then washed under running water. Then, the slide was washed with ethanol and then with running water. Safranin was used to flood the slide and left to stand for about 30 s. The slide was then rinsed under running water and left to dry. Dried slides were viewed at X100 oil immersion magnification under a light microscope; violet stained bacteria were identified as Gram positive while pink/red stained bacteria were identified as Gram negative and their sizes, arrangement and shapes were also determined

Plate 3.3: Gram staining of bacterial species

3.4.2.2. Oxidase Test

The test was used to identify isolates that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain.

A colony from a fresh culture on BA was picked with a sterile cotton swab and then a drop of oxidase reagent was dropped on it. After 1 min. a blue-purple colour indicates the presence of cytochrome c oxidase and which also means the bacteria had oxidized the reagent (tetramethyl-p-phenylenediamine) to (indophenols) producing the blue-purple colour end product whilst a negative reaction shows no colour change, hence the reagent remains colourless. The result obtained for this test was also used for the reading of the API 20E test.

3.4.2.3. Isolate Identification by Analytical Profile Index 20E (API 20E)

The API 20E strip consisting of 20 microtubes containing dehydrated substance was used for the identification of bacteria belonging to the family Enterobacteriaceae and other non-fastidious gram-negative rods. The dehydration substance was reconstituted with bacterial suspended in 0.85% saline and incubated for 18-24 h period. During incubation, metabolic reaction produces colour change which is either spontaneous or revealed by the addition of more reagents.

Firstly, the incubation tray was prepared by distributing 5mL of distilled water into the honey-combed wells of the tray to create a humid atmosphere for bacterial growth. One or two colonies from an 18-24 h period bacterial growth on BA were suspended in 0.85% saline which was comparable to 0.5 McFarland standard. A sterile pipette was then used to distribute the suspended bacterial growth into the microtubes in the API 20E strip (avoiding any bubble formation at the base of the microtubes).

Microtubes labeled ADH (arginine), LDC (lysine), ODC (ornithine), H_2S (Sodium thiosulphate) and URE (urea) which required anaerobic environments were created by overlaying the cupules with mineral oil. The trays were then covered and incubated at 36°C ± 2°C for 18-24 hr-period.

The results of the test were read by referring to the reading table (in appendix on page 63) and the codes generated were interpreted using the API 20E book. For the reading of test TDA ($FeCl_3$ reagent) and IND (Indole production), one drop of TDA and James reagent were added respectively. For VP test a drop of VP1 and VP2 (VP1 and VP2 are Barritt's reagent A and B) were added and reaction was read after 10 min. The result of the oxidase test was also needed to generate the code (as shown in appendix on page 63)

CHAPTER FOUR

4.0. RESULTS

4.1. DEMOGRAPHIC DISTRIBUTION OF INTERVIEWED FARMERS AT THE KORLE-BU FARMS

The results in Table 4.1 show that thirty-three (33) farmers were interviewed during the survey out of the reported total of 76 of which only one female is reported. Only male farmers were available at the time of interview. The majority of those interviewed were between 21 and 30 age range (36.36%) while those above 71 years were the least (6.06%). Majority (69.70%) of respondents interviewed had no education at all, 27.27% had at least basic education while 3% had secondary school education.

Table 4.1: Demographic Profile of Respondents Interviewed

Demography categories	Levels	Percentages (%)
Gender	Male (33)	100.00
	Female	0.00
Age distribution	10 - 20	9.09
	21 - 30	36.36
	31 - 40	18.18
	41 - 50	12.12
	51 - 60	9.09
	61 - 70	9.09
	>70	6.06
Educational level	None	69.70
	Primary	12.12
	JHS	15.15
	SHS	3.03

4.2. CROPS GROWN, FERTILIZER AND MANURE TYPE USED BY FARMERS

The results presented in Table 4.2 below show that 55% of farmers interviewed cultivated exotic crops while 15% grow indigenous crops. It was observed that 42% used organic manure for their vegetable cultivation while 12% used only inorganic fertilizers. However, quite a number of farmers (36%) used both organic and inorganic fertilizers. There were also some farmers (9%) who did not use any of the two forms of fertilizer

Table 4.2: Major Crops Grown in the Community, Fertilizer and Type of Manure Used

Major classes of crops	**Frequency**	**Percentages**
Exotic	18	55
Indigenous	5	15
Mixed	10	30
Fertilizer used		
Organic	14	42
Inorganic	4	12
Mixed (Organic + Inorganic)	12	36
None	3	9

Plate 4.1: Composted Poultry Manure with Litter at the Korle-Bu Vegetable Farms

The results presented in Table 4.3 show that about 54% of farmers interviewed used composted manure and 81% applied the manure after transplanting. Seventy-three (73%) of farmers interviewed had their last top dressing between 3-4 weeks before harvest. Fifty percent (50%) of the farmers had their mode of application of manure as ‘side placement’.

Table 4.3: Manure Management Practices on Farms

Manure management practices	**Frequency**	**Percentages**
Composted	14	53.85
Sun dried	12	46.15
Time of application		
After transplanting	21	80.77
Before transplanting	5	19.23
Top Dressing		
1 - 2 weeks	5	19.23
3 - 4 weeks	19	73.08
5 - 6 weeks	2	7.69
Mode of manure application		
Side placement	13	50.00
Broadcast	7	26.92
Ring placement	6	23.07

4.3. MICROBIAL LOAD OF MANURE FROM SELECTED FARMS

The microbial load of manure from the three farms are shown in Table 4.4. The coliform count was highest in Farm-A (2.3 x 10^5 cfu/g) and lowest in Farm-B (3.5 x 10^4 cfu/g). *E. coli* and *Salmonella* spp were detected in manure from Farm-A. (1.5 x 10^2) and Farm-C (2.3 x 10^1) respectively. Total plate count was highest in Farm-A (4.2 x10^5) but least in Farm-C (5.0 x 10^4). *S. aureus*, *B. cereus*, and *Shigella* spp were not detected in any of the manure samples analyzed. Plate 4.2 shows a representative plate with different colonies after incubation.

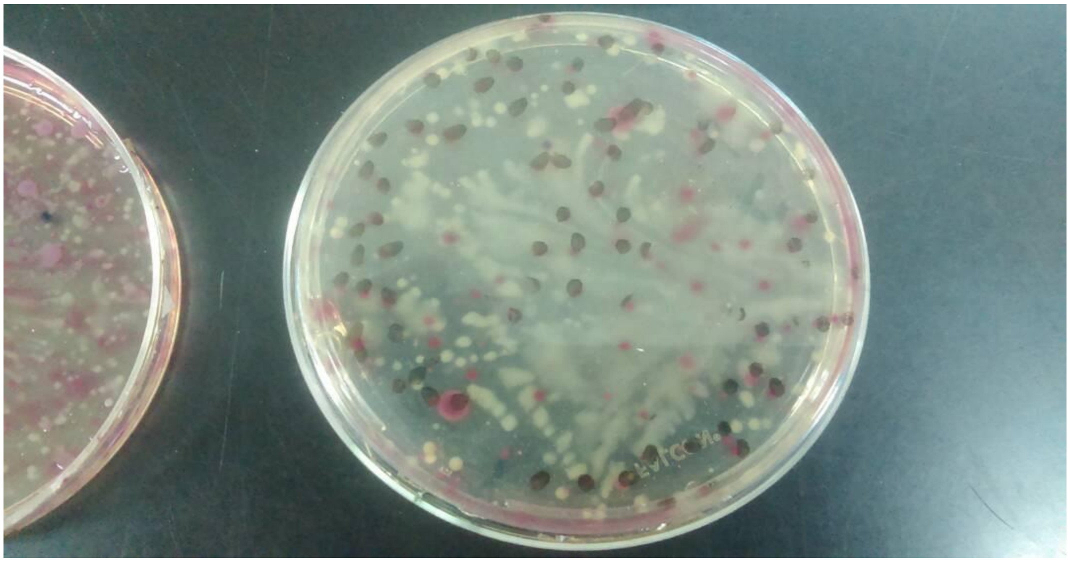


Plate 4.2: Representative plate showing Different Colonies after Incubation

Table 4.4: Microbial Load of Manure from the Selected Farms

Bacteria (cfu/g)	Farm A	Farm B	Farm C
Coliform	2.3×10^5	3.5×10^4	4.7×10^4
TPC	4.2×10^5	3.9×10^5	5.0×10^4
S aureus	-	-	-
B. cereus	-	-	-
E. coli	1.5×10^2	-	-
Salmonella spp *Shigella* spp	-	-	2.3×10^1

- absence of microbe

4.4. MICROBIAL LOAD OF LETTUCE FROM THE SELECTED FARMS

Farm-C recorded the highest coliform count of 2.0×10^3 cfu/g while Farm-B recorded the least of the coliform count of 1.3×10^3 cfu/g (Table 4.5). *E. coli* was found in lettuce from Farm-A. Total plate count was highest in Farm-C (4.5×10^3) but least in Farm-B (1.16×10^3). *S. aureus*, *B. cereus*, *Salmonella* spp, and *Shigella* spp were not detected in any of the lettuce samples analyzed (Table 4.5).

Table 4.5: Microbial Load of Lettuce from the Selected Farms

Bacteria	**Farm A (cfu/g)**	**Farm B (cfu/g)**	**Farm C (cfu/g)**
Coliform	1.4 x 10^3	1.3 x 10^3	2.0 x 10^3
TPC	2.8 x 10^3	1.16 x 10^3	4.5 x 10^3
S. aureus	-	-	-
B. cereus	-	-	-
E. coli	5.6 x 10^2	-	-
Salmonella spp	-	-	-
Shigella spp			

- absence of microbe TPC – Total Plate Count

4.5. MICROBIAL LOAD OF CARROTS FROM THE SELECTED FARMS

Farm-C recorded the highest coliform count of 2.1 x 10^3 cfu/g while Farm-B recorded the least of the coliform count of 2.2 x 10^2 cfu/g. In the case of the total plate count (TPC), the least was found in Farm-A and with the highest from Farm-B. *S. aureus*, *B. cereus*, *E. coli*, *Salmonella* spp, and *Shigella* spp were not detected in any of the carrot samples analyzed (Table 4.6). Tests were carried out in triplicates and the means recorded.

Table 4.6: Microbial Load of Carrots from the Selected Farms

Bacteria (cfu/g)	Farm A	Farm B	Farm C
Coliform	4.2×10^2	2.2×10^2	2.1×10^3
TPC	5.6×10^3	2.4×10^4	1.2×10^4
S. aureus	-	-	-
B. cereus	-	-	-
E. coli	-	-	-
Salmonella spp *Shigella* spp	-	-	-

- absence of microbe TPC = Total Plate Count

4.6. MICROBIAL LOAD OF GREEN PEPPER FROM SELECTED FARMS

From Table 4.7, green pepper from Farm-C recorded the highest coliform count of 1.5×10^3 cfu/g while that from Farm-A had the lowest of 1.0×10^3 cfu/g. Green pepper from Farm-A, however, recorded the highest total plate count (TPC) of 6.3×10^4 cfu/g, while that from Farm-C recorded the least count of 2.0×10^4 cfu/g

Table 4.7: Microbial Load of Green Pepper from Selected Farms

Bacteria (cfu/g)	Farm A	Farm B	Farm C
Coliform	1.7×10^2	1.0×10^3	1.5×10^3
TPC	6.3×10^4	4.2×10^4	2.0×10^4
S. aureus	-	-	-
B. cereus	-	-	-
E. coli	-	-	-
Salmonella spp *Shigella* spp	-	-	-

- represents microbe was not present, TPC = Total Plate Count

CHAPTER FIVE

5.0. DISCUSSION

Findings on farmers’ demographic and good manure practices have been discussed.

At the time of survey, thirty-three (33) farmers out of the reported seventy-six (76) were present on the farms. The farmers expressed that lack of funds and inputs, unfavourable weather conditions for certain crops as well as travelling (most farmers were migrants from neighboring countries) may be some of the reasons for the low number of farmers at the time of the survey. The ages of most of the farmers were between 21 and 40 years denoting the fact that vegetable farming at the Korle-Bu farms is mostly associated with the youth which is forms the main and majority of the work force at many work organizations. Majority (70%) of respondents interviewed had no education at all. Low educational levels were also reported by Agyarko and Adomako (2007), with Sisala district recording as high as 55% illiteracy levels among farmers. In their report, higher proportions of educated farmers in Shama (55%) and Birim (75%) districts were attributed to the fact that southern Ghana had higher proportion of literates compared to northern Ghana (World Development Report 2006). The high proportion of illiterate farmers at the Korle-Bu farms in this study, which is located in the south of Ghana deviates from this trend. This could however be explained from the fact that the farmers were not indigenes from southern Ghana but mostly migrants from other countries.

From the survey conducted, it was observed that majority of the respondents used organic manure for their vegetable cultivation while others used only inorganic fertilizers. However, quite a number of farmers used both organic and inorganic fertilizers and some did not use any of the two forms of fertilizer. They resorted to inorganic fertilizers when the organic was in short supply. Majority of respondents cultivated exotic crops while others cultivated indigenous. Some farmers cultivated both exotic and indigenous crops.

Many consumers patronize salads made from these vegetables from street food vendors and restaurants in the urban areas and this could account for their high cultivation in urban farms.

Although organic farming is an eco-friendlier farm practice, since it excludes chemicals and antibiotics (Reganold *et al*.,2001) and presents more profitable, environmentally sustainable and energy efficient farming system than the conventional system the preference for organic fertilizers by the respondents was largely based on its cheaper cost compared to inorganic fertilizers.

Fifty-four percent (54%) of farmers interviewed preferred to use manure which is composted while 46% preferred to use the sun-dried manure. According to Johnson *et al*., (1995) both composting and sun drying may result in heat generation in the manure, which might end up destroying or inhibiting the growth of some pathogenic microorganisms.

Considering the time of application of manure on the farms, 81% of the farmers indicated they applied the manure after transplanting which is not a good agronomic practice. While 19% applied it before transplanting. Fifty (50%) of the farmers side placed the manure while 27% broadcasted the manure on the farm with 23% respondents ring placed to apply the manure. Applying manure after transplanting may end up contaminating the crop/vegetable if the manure is contaminated. In this case, the manure may end up on the plants and if the conditions are conducive will cause microorganisms to thrive. The time allowed before transplanting after application of the manure may allow the sun’s heat and UV radiations to kill most of the pathogenic microbes before the transplanting is done.

From results of the questionnaire administration, it could be inferred that the risk of contamination of vegetables grown with animal manure is minimal considering the modes of application practiced by the farmers interviewed in this study. Broadcasting (by 27% of farmers) the manure on the farm could cause the manure to come in contact with the crops, thereby contaminating them, while applying the manure around the crop also poses a risk of contact with the crops, especially with crops that are closer to the soil (Gagliardi and Karns, 2002)

Seventy -four percent (74%) of the famers waited for 3 to 4 weeks after application of the manure before harvest, whereas 19% harvested a week or two after application of manure (Table 4.3). About eighty one percent (81%) waited for a relatively longer time (5 to 6 weeks) before harvesting the crops. It has been reported by Ingham *et al*. (2004), that allowing an interval of greater or equal to 120 days (>17 weeks) between manure application and harvest can drastically reduce the microbial levels. However, since most of the vegetables are ready for harvest after a few weeks, it is important that manure applied on vegetable farms be treated to eliminate pathogenic microorganisms before application on farms.

According to Bowman (2009) and Pappas *et al*. (2008), a myriad number of viruses, bacteria and parasites which are known to be pathogenic can be found in the faeces of some domestic animals. The faeces of these domestic animals such as poultry, are used as manure in some vegetable growing farms. The pathogenic bacteria found in the faecal matter may include *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., *Bacillus anthraxis*, and others. *E. coli* and *Salmonella* that were reported in the manure, in this study, could find their way onto the crops cultivated. This could contaminate the crops and pose some health problems to consumers of these crops. Since vegetables such as carrots, green pepper and lettuce are ready-to-eat produce, the impact on the health of consumers is usually high in the case of contamination.

Exposure of manure to sunlight or heat during composting is able to also inhibit the growth of pathogenic microbes (Sobsey *et al*. 2006) and this might account for the reason why the manure was not loaded with pathogenic bacteria.

Observing the trend of microbial load in the manure used on the three (3) selected farms it could be observed that the microbial load in the manure might have had some influence on the microbial contamination of the lettuce. The *E. coli* in the manure from Farm-A could have been transferred to the lettuce from Farm-A. *Salmonella* spp., and *Shigella* spp. were not detected in any of the lettuce from the three farms. However, all the microbial loads were within the WHO standard for these vegetables of an acceptable limit of 1.0×10^{5}.

TPC count and coliform levels reported in this study are much lower than that reported by Guchi and Ashenafi (2010), with TPC count of lettuce of 3.2×10^{7} cfu/g and coliform count of 9.3×10^{4} cfu/g from their study. This could be attributed to the type of manure used, the type of soil, and other environmental factors. Certain factors affect how long microorganisms are able to survive in soils. For example, in soils with low pH, acidophiles thrive well while high pH favours alkalinophiles, and oxygen limitation either enhances or suppresses some organisms. Water activity and the composition of the soil which will form substrate of the organism too may affect their survival. Environmental field factors surrounding the site in the form of solar radiation and dryness may also affect the survival of microorganisms (Tamasi, 1981 cited in Xiuping *et al*., 2002). This could explain the lower levels of microbial load in the lettuce samples although higher levels were recorded in the manure the farmers applied. The differences in the different microbial counts on the lettuce from the different farms are likely to be due to the differences in the soil composition and the field environmental conditions. (Tamasi, 1981 cited in Xiuping *et al*., 2002).

According to ICMSF (1996), Total Plate Count (TPC) levels up to 10^3 are acceptable, counts of 10^4 to 10^5 are tolerable, and counts of 10^6 and above are unacceptable.

From the study, although manure had relatively higher microbial loads the lettuce harvested cultivated with it had tolerable levels of TPC and coliforms. Considering that the vegetables were not washed prior to testing in the lab, the type and pH of soil and irrigation water, solar radiation, and manure application practices among others could have accounted for the lower microbial loads on the lettuces harvested from the farms. The levels recorded for coliform count were similar to that obtained by Guchi and Ashenafi (2010), who recorded 1.1×10^4 cfu/g; however, the results for TPC in this study was lower than 3.4×10^7 cfu/g reported by Guchi and Ashenafi (2010). Ibenyassine et al. (2007), in their study on ‘bacterial pathogens recovered from vegetables irrigated by wastewater in Morocco’ also recorded TPC ranging from 10^1 to values greater than 10^8 for green pepper.

Green pepper fruits are often higher away from the soil, as compared with lettuces which sometimes touch the soil and carrots which are buried in the soil on which the manure is applied. The TPC on the green pepper from farm A was highest. The levels recorded could have also been due to some other factors such as water used for irrigation and other environmental practices. From this study, the microbial loads on carrots were tolerable. Carrots are buried in the soil and as such microrganisms from manure could not be found on them.

TPC from all farms were within the acceptable range reported for ready-to-eat foods by WHO standard.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Although farmers seemed not to have any education their manure management techniques was generally good except for application of manure after transplanting which is not a good agronomic practice.

From the study, the highest Total Plate Count (TPC) of the poultry manure from the three farms was 4.2 x10^5 cfu/g with the highest coliform count being 2.3 x 10^5 Cfu/g. TPC of lettuce ranged from 2.8 x 10^3 to 1.16 x 10^3 cfu/g and coliform count from 1.3 x 10^3 to 2.0 x 10^3 cfu/g. TPC of carrots ranged from 5.6 x10^3 cfu/g to 2.4 x 10^4cfu/g and coliform count was 2.2 x 10^2 to 2.1 x10^3 cfu/g. Green pepper had TPC ranging from 2.0 x 10^4 to 6.3 x 10^4 cfu/g and coliforms from 1.0 x 10^3 cfu/g to 1.5 x 10^3 cfu/g.

All microbial counts for the vegetables were within tolerable ranges for ready-to-eat foods. After harvesting one can use either vinegar or salt to further reduce the microbial load before consumption of such produce.

E. coli and *Salmonella* spp. were detected in the manure.

6.2. RECOMMENDATIONS

It is highly recommended that the water for irrigation which is usually from drains should be assessed in further studies so that a holistic picture on contamination of the vegetables on the farms be ascertained. Also, further studies may look at the microbial quality of ready-to-eat salads from eateries.

REFERENCES

Agyarko, K., and Adomako, W. J. (2007). Survey of the use of organic manure among vegetable farmers in selected districts in Ghana. *Journal of Sustainable Development in Africa*, **9**(4).

Allos, B. M., and Taylor, D. N. (1998). Campylobacter infections. In *Bacterial Infections of Human*. Springer US. Pp. 169-190.

Amoah, P., Drechsel, P., Henseler, M. and Abaidoo, R. C. (2007) Irrigated urban vegetables production in Ghana: microbiological contamination in farms and markets and associated consumer risk groups. *Journal of Water and Health*, **5**(3): 455-466.

Beuchat, L.R. (1995) Pathogenic microorganisms associated with fresh produce, *Journal. Food Prot*ection, **59**: 204-216.

Boehm, R. (1996). Auswirkungen von ru¨ cksta¨nden von antiifektiva in tierischen ausscheidungen auf die gu¨ llebehandlung und den boden. Dtsch. Tieraerztl. Wochenschr. **103**:264–268.

Bowman, J. (2009). Manure pathogens: manure management, regulations, and water quality protection. Water Environmental Federation, McGraw-Hill, New York. Pp. 562.

Buswell, C. M., Herlihy, Y. M., Keevil, C. W., and Leach, S. A. (1999). The effect of aquatic biofilm structure on the survival of the enteric pathogen *Campylobacter jejuni* in water. *Special Publication-Royal Society of Chemistry*, **242**(1): 153-159.

Chen, Z., and Jiang, X. (2014). Microbiological safety of chicken litter or chicken litter-based organic fertilizers: a review. *Agriculture*, **4**(1): 1-29.

Collins, C. I., E. A. Murano, and I. V. Wesley. (1996). Survival of *Arcobacter butzleri* and *Campylobacter jejuni* after irradiation treatment in vacuum-packaged ground pork. *Journal of Food Protection*, **59**(11): 1164-1166.

Cornish, G A. and P. Lawrence. (2001). Informal irrigation in peri-urban areas: A summary of findings and recommendations, DFID's Water KAR Project R7132, Report OD 144, HR Wallingford, UK, pp. 54.

Cornish, GA, J.B. Aidoo, and I. Ayamba. (2001). Informal irrigation in the peri-urban zone of Kumasi, Ghana. An analysis of farmer activity and productivity. Report OD/TN 103, February 2001. DFID's Water KAR Project R7132, HR Wallingford, UK, pp.39.

De Liguoro, M., V. Cibin, F. Capolongo, B. Halling-Sorensen, and C.Montesissa. (2003). Use of oxytetracycline and tylosin in intensive calf farming: Evaluation of transfer to manure and soil. *Chemo-sphere*, **52**: 203–212.

Donkor ES, Lanyo R, Kayang BB, Quaye J. and Edoh DA (2010) Internalization of microbes in vegetables: microbial load of Ghanaian vegetables and the relationship with different water sources of irrigation. *Journal of Biological Science*. **13**(17): 857-861.

EPA-OW (2013) Literature Review of Contaminants in Livestock and Poultry Manure and Implications for Water Quality, pp. 14.

Falomir, P., Gozalbo, M. and Rico, D. (2010) Coliform bacteria in fresh vegetables: From cultivated lands to consumers Current Research, Technology and Education topics in Applied microbiology and microbial biotechnology. 2

Froseth, R.B., Johannessen, G., Solemdal, L., Jarp, J., Wasteson, Y. and Rorvik, L.M. (2005) Influence of cattle manure on the bacteriological quality of organic Iceberg lettuce. *Journal of Applied Microbiology* 96:787-794

Gagliardi, J.V. and Karns, J.S. (2002). Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. *Environmental Microbiology*, **4**: 89-96.

Gil, J. M., Gracia, A. and Sanchez, M. (2000). Market Segmentation and Willingness to pay for organic products in Spain‖. *International Food and Agribusiness Management Review* **3**: 207-226.

Golly M.K., Salifu P.S., Mills-Robertson F.C. (2016). Resistance of Bacteria Isolates from Cabbage (*Brassica oleracea*), Carrots (*Daucus carota*) and Lettuce (*Lactuca sativa*) in the Kumasi Metropolis of Ghana. *International Journal of Nutrition and Food Sciences*. **5**(4): 297-303. doi: 10.11648/j.ijnfs.20160504.20.

Guchi, B. and Ashenafi, M. (2010). Microbial load, prevalence and antibiograms of *Salmonella* and *Shigella* in lettuce and green pepper. *Ethiopian Journal of Health Sciences*, 20(1).

Hasan, M. R. and Solaiman, A.H.M. (2012). Efficacy of organic and organic fertilizer on the growth of *Brassica oleracea* L. (Cabbage). *International Journal Agri Crop Science,* **4**(3): 128-138.

Hedberg, C. W., Angulo, F. J., White, K. E., Langkop, C. W., Schell, W. L., Stobierski, M. G., .and Griffin, P. M. (1999). Outbreaks of salmonellosis associated with eating uncooked tomatoes: implications for public health. *Epidemiology and Infection*, **122**(03): 385-393.

Hoorman, J.J., Rausch J. N. and Brown, L. C. (2008), Guidelines for Applying Liquid Animal Manure to Cropland with Subsurface and Surface Drains, Agriculture and Natural Resources Fact sheet, Ohio State University ANR-21-09

ICMSF **(**1996). International Commission on Microbiological Specifications for Foods ICMSF, Microorganisms in Foods 5: Microbiological Specifications of Pathogens.

Ingham, S. C., Losinski, J. A., Andrews, M. P., Breuer, J. E., Breuer, J. R., Wood, T. M., and Wright, T. H. (2004). *Escherichia coli* contamination of vegetables grown in soils fertilized with non-composted bovine manure: garden-scale studies. *Applied and environmental microbiology*, **70**(11): 6420-6427.

Islam, M. Doyle, M.P. Phatak, S.C. Millner, P. Jiang, X. (2004). Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water, *Journal.of Food Prot*ection, **67**: 1365-1370.

Johannessen, G.S., R.B. Frøseth, L. Solemdal, J. Jarp, Y. Wasteson and L. M. Rørvik. (2004). Influence of bovine manure as fertilizer on the bacteriological quality of organic Iceberg lettuce. *Journal ofApplied Microbiology*, **96**: 787-794.

Johnson, J., and Eckert, D., (1995), Best management practices: Land application of animal manure. OhioState University Extension Publication AGF-208- 95 http://www.ag.ohio state. edu/~ohioline/agf fact/0208.html (Accessed on May 2015)

Khandaghi, J., Razavilar, V., and Barzgari, A. (2010). Isolation of Escherichia coli O157: H7 from manure fertilized farms and raw vegetables grown on it, in Tabriz city in Iran. *African Journal of Microbiology Research*, **4**(9): 891-895.

Kipkosgei, L.K. Akundabweni L.S.M. and Hutchinson M.J., (2003). The effect of farmyard manure and nitrogen fertilizer on vegetative growth, leaf yield and quality attributes of *Solanum villosum (*Black nightshade) in Keiyo district, rift valley, African Crop Science Conference Proceedings, **6**: 514-518.

Kumar, K., Thompson, A., Singh, A. K., Chander,Y. and Gupta, S.C. (2004). Enzyme-linked immunosorbent assay for ultratrace determination of antibiotics in aqueous samples. *Journal on Environmental. Quality.* **33**: 250–256.

Kumar, K., Gupta, S. C., Baidoo, S. Chander, K. Y. and Rosen, C. J. (2005). Antibiotic Uptake by Plants from Soil Fertilized with Animal Manure. *Journal on. Environmental. Quality*. **34**: 2082–2085.

Levy, S.B., and Marshall, B., (2004) Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine Supplement,* **10**(12): S122-S129.

Li, X., Payne, J.B., Santos, F.B., Levine, J.F., Anderson, K.E., Sheldon, B.W. (2007). *Salmonella* populations and prevalence in layer feces from commercial high-rise houses and characterization of the *Salmonella* isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. *Poultry Science,* **86**: 591–597.

Lohr, L. (1998). Implications of Organic Certification for Market Structure and Trade. *American Journal of Agricultural Economics,* **80**(5):1125-1129.

Loncarevic, S., Johannessen, G. S., and Rørvik, L. M. (2005). Bacteriological quality of organically grown leaf lettuce in Norway. *Letters in Applied Microbiology,* **41**(2): 186-189.

Lopez-Real, J. M. (1995) Organic Wastes and Peri-urban Horticulture, Visit to Kumasi, Ghana.

Machado, D. C., Maia, C. M., Carvalho, I. D., Silva, N. F. D., André, M. C. D. P. B., and Serafini, Á. B. (2006). Microbiological quality of organic vegetables produced in soil treated with different types of manure and mineral fertilizer. *Brazilian Journal of Microbiology,* **37**(4): 538-544.

Masarirambi, M.T., Hlawe, M. M. Oseni, O. T. and Sibiya T. E. (2010) Effects of organic fertilizers on growth, yield, quality and sensory evaluation of red lettuce (*Lactuca sativa* L.) ‘Veneza Roxa’. *Agric. Biol*ogy **1**(6): 1319-1324.

Mason, M. J., Humphrey, T. J., and Martin, K. W. (1999). Isolation of sublethally injured campylobacters from poultry and water sources. *British journal of biomedical science,* **56**(1): 2.

McEwen, S.A., and Fedorka-Cray P.J (2002). Antimicrobial use and resistance in animals. *Clinical Infectious Diseases* **34** (Suppl. 3): S93–S106.

McMahon, M.A.; Wilson, I.G. (2001). The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *International Journal Food Microbiology*, **70**: 155-162.

Mensah, E., Amoah, P., Abaidoo, R.C. and P. Drechsel. (2001). Environmental concerns of (peri-) urban vegetable production – Case studies from Kumasi and Accra. In: Drechsel, P. and D. Kunze (eds.) Waste Composting for Urban and Peri-urban Agriculture - Closing the rural-urban nutrient cycle in sub-Saharan Africa. IWMI/FAO/CABI: Wallingford, pp. 55-68.

Ministry of Health and Welfare of Japan (1997). Verocytotoxin-producing *Escherichia coli* (enterohemorrhagic *E. coli*) infection, Japan 1996-June 2007, National Institute of Infectious Diseases and Infectious Disease Control Division, Infectious Agents Surveillance Report, **18**:153-154.

Mougeot, L. J. (2000). Urban agriculture: definition, presence, potentials and risks. *Growing cities, growing food: Urban agriculture on the policy agenda*, pp. 1-42.

Mukherjee, A., Speh, D., Dyck, E., and Diez-Gonzalez, F. (2004). Preharvest evaluation of coliforms, *Escherichia coli*, *Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *Journal of Food Protection®*, **67**(5): 894-900.

Natvig, E.E., Ingham, S.C., Ingham, B. H., Cooperband, L.R. and Roper, T.R. (2002). *Salmonella enterica* serovar *typhimurium* and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. *Applied and Environmental Microbiology*, **68**: 2737-2744.

Nouhoheflin, T., Coulibaly, O.,Andy, J., Cherry, Al-Hassan and Patrice Y. (2004) Consumers' Perception and Willingness to Pay for Organic Vegetable in Benin and Ghana. *Shaping the Future of African Agriculture for Development: The Role of Social Scientists*. Proceedings of the Inaugural Symposium, 6 to 8 December 2004, Grand Regency Hotel, Nairobi, Kenya. http://ageconsearch.umn.edu/bitstream/9525/1/cp04no01.pdf

Obeng-Ofori, D., Danquar, E.Y., Ofosu-Anim, J. (2007). Vegetable and Spice Crop Production in West Africa, The City Publishers Limited, Ghana. Pp. 77-79.

Obuobie, E., Keraita, B., Danso, G., Amoah, P., Cofie, O.O., Raschid-Sally, L. and P. Drechsel. (2006). Irrigated urban vegetable production in Ghana: Characteristics, benefits and risks. IWMI-RUAF-CPWF, Accra, Ghana: IWMI, 150 pp.

Okiki, P. A., Edafiadhe, W E. and Ogbimi, A. O. (2010). Use of poultry manure in vegetable cultivation and influenza pandemic. *Advances in Applied Science Research*, **1**(3): 1-7.

Pappas, E., R. Kanwar, J. Baker, J. Lorimor, and S. Mickelson (2008). Faecal indicator bacteria in subsurface drain water following swine manure application. *American Society of Agricultural and Biological Engineers*, **51**(5): 1567-1573.

Phillips, I., M. Casewell, T. Cox, B.I. Groot, C. Friis, R. Jones, G. Nightingale, R. Preston, and J. Waddell. (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of. Antimicrobial. Chemother*apy **53**: 28–52.

Phillips, J. C. and Peterson, H. C. (2001). Demand Overview for Organic Produce. Staff Paper No. 2001-06 Department of Agricultural Economics Michigan State University, East Lansing, USA.

Reganold, J.P., Glover, J. D., Andrews, P. K. and Hinman, H. R. (2001). ―Sustainability of three Apple Production Systems. *Nature*, **410**:926-930.

Rogers, S., and J. Haines (2005). Detecting and mitigating the environmental impact of fecal pathogens originating from confined animal feeding operations: review. EPA-600-R-06-021. USEPA, Office of Research and Development, National Risk Management Research Laboratory. Cincinnati, OH.

Rosen, C. J. and Bierman, P. M., (2005). Nutrient Management for Fruit & Vegetable Crop Production- Using Manure and Compost as Nutrient Sources for Vegetable Crops, University of Minnesota Extension Service bulletin.

Sagoo, S.K.; Little C.L.; Mitchell, R.T. (2001). The microbiological examination of ready-to-eat organic vegetables from retail establishments in the United Kingdom. *Letter of Applied Microbioogy*, **33**: 434-439.

Sawyer, J. E., Mallarino, A. P., (2016). Using manure nutrients for crop production, Iowa State University Extension Outreach, PMR 1003, pp. 4.

Shiyam, J. O. and Binang W. B. (2013). Effect of poultry manure and plant population on productivity of fluted pumpkin (*Telfaiaria occidentalis* Hook F.) in Calabar, Nigeria. *Journal of Organic Systems,* **8**(2).

Slavin, J. L., and Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition: An International Review Journal*, **3**(4), 506-516.

Smit, J., Ratta, A. and Nasr J. (1996). “Urban Agriculture: Food, jobs and sustainable cities”. Publication carried for Habitat II. Volume I. New York: United Nations Development Programme (UNDP).

Sobsey, M. D., Khatib, L. A., Hill, V. R., Alocilja, E., and Pillai, S. (2001). Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate.

White Papers on Animal Agriculture and the Environment. MidWest Plan Service (MWPS), Iowa State University, Ames, IA.

Sobsey, M.D., L.A. Khatib, V.R. Hill, E. Alocilja, and S. Pillai. (2006). Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate. pp. 609-666. *In* J. M. Rice, D. F. Caldwell, and F. J. Humenik (ed.) Animal Agriculture and the Environment: National Center for Manure and Animal Waste Management White Papers. American Society of Agricultural and Biological Engineers. St. Joseph, Michigan.

Solomon, E.B.; Yaron, S.; Matthews, K.R. (2002). Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied* Environmental. *Microbiology*., 68: 397-400.

Sussman, M. (Ed.). (1997). *Escherichia coli: mechanisms of virulence*. Cambridge University Press.

Thompson, G.D. (1998) ―Consumer Demand for Organic foods: What we Know and What Need to Know. *American Journal of Agricultural Economics*, **80**(5): 1113-1118.

Thompson, S., and Powell, D. A. (2000). Risks associated with the consumption of fresh sprouts. *Food Safety Network Technical Rep*, **16**.

Tiimub, B. M., Kuffour, R. A. and Kwarteng, A. S., (2012), Bacterial Contamination Levels of Lettuce Irrigated with Waste Water in the Kumasi Metropolis. *Journal of Biology, Agriculture and Healthcare*, **2**(10): 116-127.ISSN 2224-3208

Tschäpe, H., R. Prager, W. Streckel, A. Fruth, E. Tietze and G.Böhme (1995). Verotoxinogenic Citrobacter freundii assiciated with severe gastroenteritis and cases of haemolytic ureamic syndrome in a nursery school: green butter as the infection source. *Epidemiology and Infection*, 114, 441-450.

USEPA, (2004), Risk management evaluation for concentrated animal feeding operations, EPA-600-R-04-042, USEPA, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, OH.

Uzeh, R.E. and Adepoju, A. (2013). Incidence and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on salad vegetables. *International Food Research Journal*, **20**(4): 1921-1925.

Van Duyn, M. A. S., and Pivonka, E. (2000). Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *Journal of the American Dietetic Association*, **100**(12): 1511-1521.

Veterinary Services Department (1994). *Livestock Population Census,* Ministry of Food and Agriculture, Accra, Ghana.

WHO/FAO, (2003). Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser*, **916**(i-viii): 24.

World Development Report 2006. p65

Worthington, V. (1998). Effect of agricultural methods on nutritional quality: a comparison of organic with conventional crops. *Alternative Therapies Health Med*icine, **4**(1): 58-69.

Worthington, V. (2001). Nutritional quality of organic versus conventional fruits, vegetables and grains. *Journal. Alternative Complementary*. *Medicine*, **7**: 161-173.

Xiuping, J., Morgan, J. and Doyle, M. P. (2002)**.** Fate of *Escherichia coli* O157:H7 in Manure-Amended Soil. *Appl Environ Microbiol*., 68(5): 2605–2609.

Zibrilla, I. and A.A. Salifu. (2004). Information gathering from urban and peri-urban communities with potential land areas for vegetable production. Report submitted to Urban Agriculture Network – Northern Ghana. 30th June 2004, pp. 16.

APPENDICES

APPENDIX I

Media Preparation

Plate Count Agar (PCA)

About 17.5 g of agar powder was suspended in 1 L of distilled water, boiled to dissolve the agar completely and sterilized in an autoclave at 121 ^{o}C for 15 minutes. The mixture was then cooled, poured into sterile Petri dishes and allowed to set.

E. coli / Coliform (Brilliance *E. coli*/coliform selective agar) Agar

Into about 1 L of water, 28.1 g of agar powder was suspended and boiled gently with intermittent agitation to dissolve the agar completely. The mixture was then cooled to 50 ^{o}C, poured into sterile petri dishes and allowed to set. Set Petri dishes were then stored in an incubator overnight to verify sterility.

Salmonella/Shigella Agar

About 55 g of agar powder was suspended in 1 L of distilled water. The mixture was boiled to until dissolved completely. It was then cooled, poured into sterile Petri dishes and allowed to set. Set petri dishes were then stored in an incubator overnight to verify sterility.

Blood Agar (BA)

About 39 g of agar powder was suspended in 1 L of distilled water and boiled until completely dissolved. The mixture was sterilized in an autoclave at 121 ^{o} C for 15 minutes and cooled to between 45 - 50 ^{o}C. Sterile defibrinated (non-clotted) blood was then added to the agar, thoroughly mixed. The blood agar mixture was then poured into sterile Petri dishes and allowed to set. Set petri dishes were then stored in an incubator overnight to verify sterility.

APPENDIX II

Biochemical Test (According to manufacturers' protocol)

Oxidase test

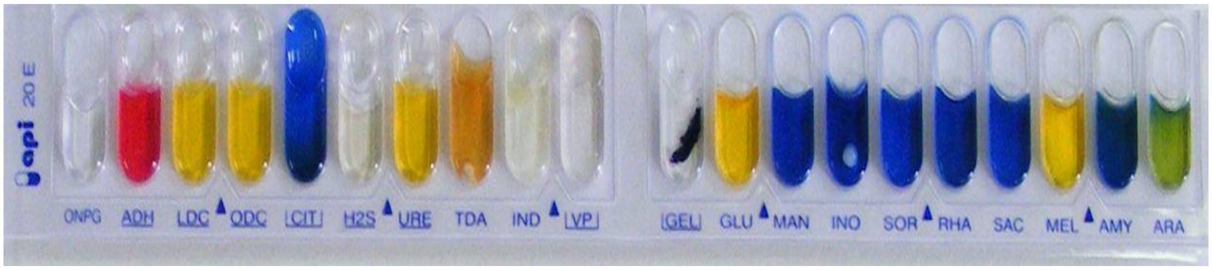
Three drops of 1% solution of tetramethyl-p-phenylene diamine dihydrochloride were put on a piece of filter paper in a Petri dish. Pure test colonies were smeared on the surface of the paper and observed for the development of blue colour on the filter paper.

Catalase test

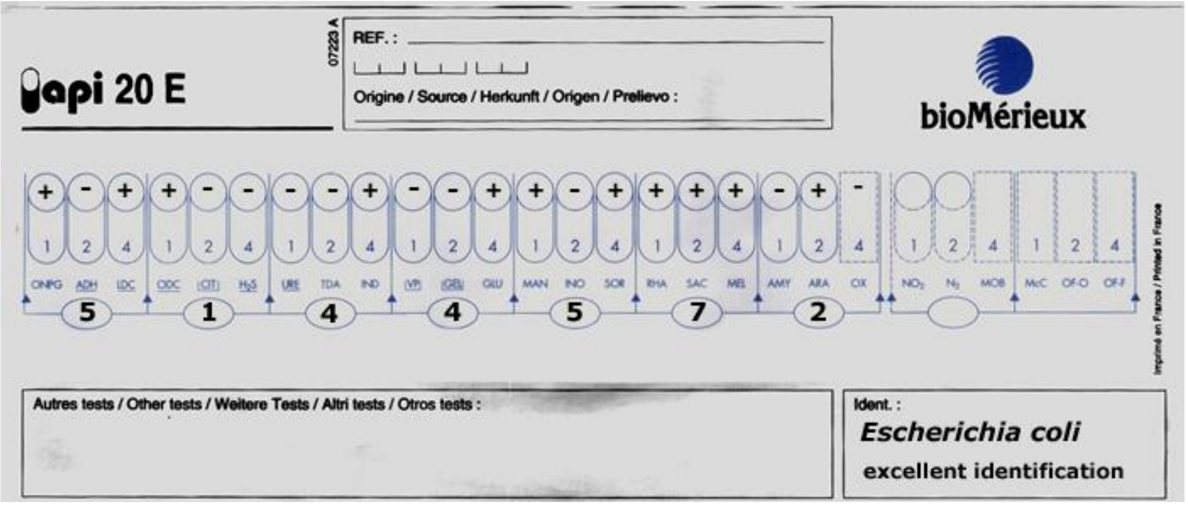
Qualitative procedure for determining catalase activity by bacteria. The enzyme is present in most aerobic and facultatively anaerobic bacteria with the major exception of streptococci and enterococci.

Table 5: Reading table for API 20E

		Results	
Test	Active Ingredient	Negative	Positive
OPNG	2-nitrophenyl-βD-galactopyranoside	colourless	Yellow
ADH	L-Arginine	yellow	Red-orange
LDC	L-Lysine	yellow	Red-orange
ODC	L-ornithine	yellow	Red-orange
CIT	Trisodium citrate	Pale green-yellow	Green-blue
H_2S	Sodium thisulfate	Colourless-greyish	Black deposit-thin line
URE	Urea	yellow	Red-orange
TDA	L-Tryptophan	yellow	Reddish-brown
IND	Indole	Colourless-pale green-yellow	Pink
VP	Sodium pyruvate	Colourless-pale pink	Pink-red
GEL	Gelatin (bovine origin)	No diffusion	Diffusion of black pigment
GLU	D-glucose	Blue-blue green	Yellow-greyish yellow
MAN	D-mannitol	Blue-blue green	yellow
INO	Inositol	Blue-blue green	yellow
SOR	D-sorbitol	Blue-blue green	yellow
RHA	L-rhamnose	Blue-blue green	yellow
SAC	D-saccharose	Blue-blue green	yellow
MEL	D-melibiose	Blue-blue green	yellow
SAC	D-sucrose	Blue-blue green	yellow



API 20E strips



api 20 E

07223 A

REF. :

Origine / Source / Herkunft / Origen / Prelievo :

bioMérieux

+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	+	+	-	+	-						
1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
ONPG	ADH	LDC	ODC	(CIT)	H_2S	URE	TDA	IND	(VP)	(GEL)	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO_2	N_2	MOB	McC	OF-O	OF-F
5			1			4			4			5			7			2								

Imprimé en France / Printed in France

Autres tests / Other tests / Weitere Tests / Altri tests / Otros tests :

Ident. :

Escherichia coli

excellent identification

A Typical Result for *E. coli*

APPENDIX III

Questionnaire

Manure management practices

Demographic Profile of farmers Interviewed

Gender

Male
Female

Age Distribution

10 - 20
21 - 30
31 - 40
41 - 50
51 - 60
61 - 70
>70

Educational level

None
Primary
JHS
SHS

Major Crops Grown in the Community, Fertilizer and Type of Manure Used

Major classes of crops

Exotic
Indigenous
Mixed

Fertilizer used

Organic
Inorganic
Mixed (Organic + Inorganic)
None

Manure Management Practices on Farms

Composted
Sun dried

Time of application

After transplanting
Before transplanting

Top Dressing

1 - 2 weeks
3 - 4 weeks
5 - 6 weeks

Mode of manure application

Side placement
Broadcast
Ring placement