KWA KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF ENVIRONMENTAL SCIENCE

EFFECTS OF AGING ON PERSISTENCE OF *Escherichia coli (E. coli)* AND COLIFORMS IN POULTRY MANURE

A THESIS PRESENTED TO THE DEPARTMENT OF ENVIRONMENTAL SCIENCE, COLLEGE OF SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE, ENVIRONMENTAL SCIENCE

BY

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DECLARATION

I hereby declare that this submission is my own work towards the Master of Science degree and that, to the best of my knowledge, it contains no material previously published by another person nor materials which have been accepted for the award of any other degree of the University, except where due acknowledgements have been made.

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DEDICATI ON

I dedicate this work to my parents, Mr and Mrs Atta Boakye and also to Josephine Kyeiwaa Owusu. This project is again dedicated to Princeford Agudzie; a course mate, brother and a friend who painfully passed on mid-way through our maters program. Though he is dead his memory shall forever remain.

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ABSTRACT

The use of poultry manure as soil amendment in peri-urban vegetable farming coupled with consumer awareness on food safety and the risk of microbial contamination call for improvement of on-farm management practices. The effects of aging on the persistence of coliforms in poultry manure was evaluated over an eight (8) week period using three (3) different treatment conditions; an Aerobin composter, Turned and Unturned windrow. Temperature, moisture content, pH, total and faecal coliforms were measured over the study period. Coliform numbers reduced by more than 90% (>90) at the end of week four for all the treatments. At the end of week eight, more than 95% reductions had been recorded in the Aerobin, Turned and Unturned windrow. Mean temperatures varied from a low 34.6^{o}C in the Aerobin to 38.1^{o}C in the turned windrow to 41.7^{o}C in the Unturned windrow. Mean moisture content in the aging material was 1.63% in the Aerobin, 2.6% in the Turned windrow and 2.9% in the Unturned windrow. Composting in the Aerobin recorded the lowest mean pH of 7.6, followed by the Turned windrow, 7.8 and the Unturned windrow 8.1. The study highlights the need for further studies into the interrelationship between temperature, moisture and pathogen reduction during aging.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

The increasing demand for poultry and their products has caused excessive manure supplies in certain areas (Georgia Agricultural Statistics Service, 1999) and has garnered great attention across the world as to how to develop safe and environmentally friendly ways to reuse such supply of manure (Liu *et al*., 2010). Internationally, one of the effective and environmentally safe ways to reuse poultry litter is its application on farm lands as organic manure once it is removed from the poultry house (Thaxton, 2003). Poultry littler is not immediately applied to farm lands once it is collected from the poultry house, however it is allowed to undergo composting - a biological process in which the organic portion of waste is allowed to decompose under carefully controlled conditions (Moor *et al.*, 1995; Fei-Baffoe, 2010).

Poultry manure is made out of raw poultry droppings and bedding materials such as sawdust, wood shavings, grass cuttings, banana leaves or rice hulls (Kisselle *et al.*, 2003), which has been composted, and the combination forms an excellent source of nitrogen (N), phosphorus (P), potassium (K) and sulfur (S) (Sloan *et al*., 2003; Kisselle *et al*., 2003) making it a preferred fertilizer by most farmers (Sloan *et al*, 2003). Poultry litter may not only be recovered unto agricultural lands as organic fertilizer (Moor *et al*., 1995) though that is the conventional practice, some amounts are used as feed supplement for ruminants (Wilkinson, 2011). The major portion of litter however serves the former purpose as relatively smaller amounts are used in the latter.

Currently, in West Africa, the recovery of poultry litter onto agricultural land has become a common practise in urban vegetable farming (Danso *et al*., 2006; Nyarko *et al*., 2006), as it improves soil nutrients and structure, and consequently boosts yield (Huang *et al*., 2010; Liu

et al., 2010). In Ghana, significant improvement in urban and peri urban vegetable farming has been observed with the use of poultry manure as perishable crops like vegetables and fruits loose both their nutritional and market value during time of transportation from rural communities to urban and peri urban cities (Danso *et al.*,2006; Nyarko *et al.*, 2006). Additionally, there is evidence that vegetable farming in urban centres offer a major opportunity for the poorest people in urban and peri urban centres to earn a living, either as producers and or as traders of vegetables, without requiring large capital investments (Schippers, 2000), attributing to the preference of poultry manure due to its cost effectiveness.

Conversely, poultry as well as other animals are known to contain bacteria that have the potential to cause human illness, such as Salmonella and Staphylococcus (Martin *et al*., 1998; Himathongkham, 2000), which are shed in their faeces that make up the manure, facilitating horizontal transmission. This has caused concern in connection with the practice of applying poultry manure as a fertilizer for growing produce for human consumption; as it may result in disease (Himathongkham, 1998). The use of poultry litter in commercial vegetable farming therefore presents a potential threat to consumers. Heightened consumer awareness of food safety issues then calls for the scrutiny of on-farm management practices to reduce microbial loads since contaminated manure (Pote *et al*., 2003) is a probable vehicle for the pathogens to get to consumers to cause disease outbreaks (Wilkinson, 2011).

In the production of poultry manure , the combining effects of two distinct but similar processes are theoretically responsible for the microbial quality of the compost, namely, the Composting process itself and Maturation (aging), and the effectiveness of these processes depend on

temperature, moisture and pH and to a higher extent the feedstock for the composting process (Hartel, 2000)

High temperature is the most frequently studied mechanism involved in the inactivation of human pathogens during organic waste treatment processes such as composting and deep stacking (Thaxton, 2003). Other mechanisms such as, microbial antagonism (including antibiotic production and direct parasitism), ageing, production of organic acids, pH changes, desiccation, exposure to ammonia, and competition for nutrients (Epstein, 1997; Chaudhry *et al*., 1998) are also known to reduce pathogens. However, composting of poultry litter is not carefully monitored to ensure the effectiveness of these mechanisms in sanitizing the litter as manure production may not be the primary motive of the poultry farmer. Aging of poultry litter must therefore be monitored carefully to ensure inactivation of pathogenic microorganisms.

There is therefore a need to study the effects of different aging methods of poultry manure, establishing a relationship between the method used and reduction in microbial numbers. In this study, poultry litter was allowed to age under three different conditions; open windrows, aerobin and unturned windrow and their effects on microbial numbers assessed.

1.2. PROBLEM STATEMENT

Methods of collection, storage, and use of poultry manure is undergoing increased scrutiny in recent times because of increases in manure quantities (from increases in poultry production) and heightened environmental awareness concerning adverse effects of manure on human health and environment safety (Hartel, 2000)

Poultry litter is known to contain microorganisms that have the potential to cause human disease, such as Salmonella, Staphylococcus and Campylobacter (Martin *et al.*, 1998; Terzich,

et al., 2000) hence food poisoning outbreaks can be attributed to contamination of fresh produce by poultry manure (Brackett, 1999; Doyle and Erickson, 2008). Hatel *et al*., (2000) reported that 10 out of the 20 samples of fresh poultry manure that had not undergone aging contained significant numbers of faecal coliforms when analysed. An indication that poultry manure contains pathogenic microorganisms.

Consumer awareness of food safety issues on the other hand, has called for the need to look out for improved 'on - farm management practices' to reduce the risk of microbial contamination resulting from poultry manure usage.

Moreover, there exist substantial evidence that indicates pollution of water bodies by runoff from farms fertilized with poultry litter (Pote *et al*, 2003). Poultry and vegetable farmers may be implicated when fecal coliforms are found in surface waters following runoff events from poultry litter-amended pastures and hayfields (Hartel *et al*., 2000) as coliforms are indicator organisms used in water quality determination.

1.3 JUSTIFICATION

Wilkinson *et al*., (2011), studied the effects of simple on-farm management practices on microbiological safety of poultry litter in Australia by examining the effects of heat and moisture on the survival of Escherichia coli and Salmonella in poultry litter during aging under laboratory conditions. Although their work showed an improved microbial quality, the conditions under which their study was conducted was strictly laboratory based as higher temperatures of 60-70^{o}C were artificially set and maintained throughout the study. However, it may be difficult attaining such high temperatures during aging naturally. This study is based on field trials, otherwise giving a fair indication of what the natural aging process may be.

A ready-to-use poultry manure may have gone through two similar but distinct processes, namely, actual composting (rapid degradation phase) and aging, the latter may be referred to as maturation in some literature. The rapid degradation phase takes place in the poultry pen whilst aging normally takes place on the vegetable farms where the manure is applied. Since the primary aim of the poultry farmer is not manure production, the degradation is not monitored to ensure its microbial quality. Such factors like high temperature which is needed to sanitize the manure may interfere with poultry production and may not be advantageous to the poultry farmer. Efforts must be taken to work on the microbial quality of the manure during aging. This study therefore explores the impacts of different aging methods used in poultry manure processing on some parameters such as temperature, pH and moisture and the persistence of coliforms during aging.

1.4 OBJECTIVE

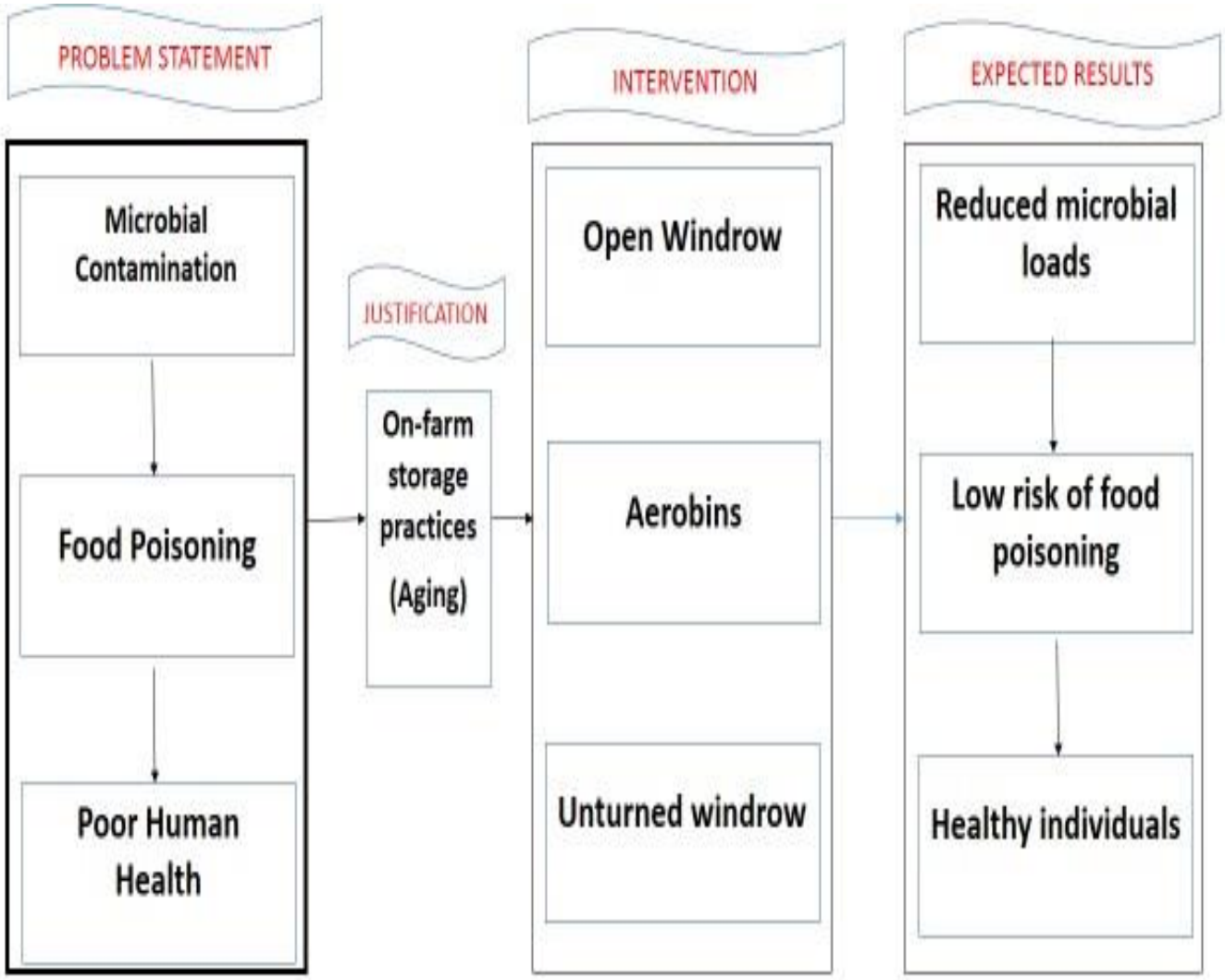
1.4.1 Main objective

To investigate the persistence of coliforms and *E. coli* in poultry manure during aging.

1.4.2 Specific objectives

1. To investigate the dynamics of physicochemical parameters (temperature, moisture and pH) during aging of poultry manure in selected methods of aging.
2. To monitor the population dynamics of coliforms and *E. coli* during aging
3. Determine the levels of N, P, K in manure before and after aging.

1.5. CONCEPTUAL FRAMEWORK OF STUDY


PROBLEM STATEMENT
Microbial Contamination
Food Poisoning
Poor Human Health
JUSTIFICATION
On-farm storage practices (Aging)
INTERVENTION
Open Windrow
Aerobins
Unturned windrow
EXPECTED RESULTS
Reduced microbial loads
Low risk of food poisoning
Healthy individuals

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 COMPOSTING

Composting is the biological decomposition of biodegradable organic fraction of waste under controlled conditions to a state sufficiently stable for nuisance free storage and handling and for safe use in land preparation (Fei-Baffoe, 2010). It requires optimizing the conditions for the mixed population of microorganisms mainly, bacteria, fungi and actinomycetes responsible for the decomposition. These microbes, normally found on the surface of leaves, grass clippings and other organic materials, thrive in a warm, moist, aerobic (oxygen rich) environment (Christian *et al.*, 2009).

Composting primarily started as backyard composting where household waste was dumped in dug holes at backyards with the intention of using land for backyard farming. With time, more effective ways of composting such as heaping was introduced. Such practise as heaping waste speeds up the natural process of decomposition as optimum conditions for decomposing microorganisms are met (Wong and Lin, 2002).

The activities of microorganisms in organic matter degradation are affected by factors such as temperature, moisture, particle size, pH and volume. Yet again the value of the compost produced depends basically on the nature of feed stock used in the process. Ensuring an optimal balance between these factors ensures both high fertilizer value and microbial quality (Wichuk *et al.*, 2007).

2.1.1. COMPOSTING AND AGING (MATURATION)

A ready to used compost goes through two major phases, namely, rapid degradation phase (actual composting) and slow degradation phase (aging) (Wilkinson *et al.*, 2011; Christian *et al*. 2009). A compost pile as decomposition takes place rises in temperature with time. However at some point the temperature begins to drop. The drop in compost pile temperature is not a sign that composting is complete, but rather an indication that the compost pile is entering another phase of the composting process called aging or maturation (Christian *et al*., 2009). During aging, mesophilic microorganism take over from heat loving microorganisms to continue the degradation process. Degradation is relatively slow and hence all other parameters are minimal.

The two phases are similar but distinct. In the case of poultry manure, the actual composting (rapid degradation) takes place in the poultry pen. Poultry dropping and bedding materials normally saw dust form the feed stock on which decomposers act to produce the manure (Sloan *et al*., 2003; Kisselle *et al*., 2003). Poultry litter stay in the pen for 12- 16 weeks before it is removed and sold to vegetable farmers as manure.

Aging most often takes place on vegetable farms where the manure is applied. On collection from the poultry farm, the manure is not readily applied to vegetable farms, farmers store manure for some time to age before its final application to farm land (Nyarko *et al*., 2006).

2.2.0. MICROORGANISMS IN A COMPOST PILE

Microorganisms in a compost pile can be grouped into classes, namely Useful microorganism and Pathogenic microorganism. Useful microorganism are the microorganism that are responsible for decomposition of organic matter to produce compost (Christian *et al*., 2009).

Pathogenic microorganism on the other hand are the microorganism that can cause human illness (Martin *et al.*, 1998). In the composting and aging process, step will have to be taken to optimise the activities of useful microorganism while eliminating pathogenic microorganism (Himathongkham, 2000).

Useful microorganisms belonging to bacteria, fungi, and actinomycetes account for most of the decomposition that takes place in a pile (Epstein *et al.*, 1976).They are considered chemical decomposers, because they change the chemistry of organic wastes through mineralisation processes. However, the composting process is not only achieved by the action of microorganism, macroorganism otherwise called Physical decomposers also play major roles by grinding, biting, sucking, tearing, and chewing materials into smaller pieces. Such physical decomposers include, snails, millipedes, springtails, spiders, slugs, beetles, ants, flies, nematodes, flatworms, rotifers, and earthworms (Kowalchuk *et al.*, 1999).

2.2.1. USEFUL BACTERIA IN COMPOST PILE

Bacteria are nutritionally diverse of all organisms and can degrade nearly all substances. Bacteria utilize carbon as a source of energy and nitrogen to build protein in their bodies. They obtain energy by oxidizing organic material, especially the carbon fraction. This oxidation process heats up the compost pile from ambient air temperature. If proper conditions are present, the pile will heat up fairly rapidly due to bacteria consuming readily decomposable materials (Liang *et al*., 2003).

While bacteria can act on a wide variety of organic compounds, they may be limited by unfavorable conditions due to their size and lack of complexity. Changes in oxygen, moisture, temperature, and acidity may affect microbial activity. Aerobic bacteria need oxygen levels

greater than five percent. They are the preferred organisms, because they provide the most rapid and effective composting. They also excrete plant nutrients such as nitrogen, phosphorus, and magnesium. When oxygen levels fall below five percent, the aerobes die and decomposition slows by as much as 90 percent (Kuter *et al.*, 1985). Anaerobic microorganisms take over and, in the process, produce a lot of useless organic acids and amines which are smelly, contain unavailable nitrogen and, in some cases, are toxic to plants. In addition, anaerobes produce hydrogen sulfide, cadaverine, and putrescine (Kuter *et al.*, 1985).

There are different types of aerobic bacteria that work in composting piles. Their populations will vary according to the pile temperature (Ohtaki *et al.*, 1998).

2.2.2. PSYCHROPHILIC BACTERIA

Psychrophilic bacteria work in the lowest temperature range. They are most active at 55° F and will work in the pile if the initial pile temperature is less than 70º F. They give off a small amount of heat in comparison to other types of bacteria. The heat they produce is enough however, to help build the pile temperature to the point where another set of bacteria, mesophilic bacteria, start to take over (Kuter *et al.*, 1985).

2.2.3. MESOPHILIC BACTERIA

Mesophilic bacteria rapidly decompose organic matter, producing acids, carbon dioxide and heat. Their working temperature range is generally between 70º to 100º F. When the pile temperature rises above 100º F, the mesophilic bacteria begin to die off or move to the outer part of the heap (Liang *et al.*, 2003). They are replaced by heat-loving thermophilic bacteria.

2.2.4. THERMOPHILIC BACTERIA

Thermophilic bacteria thrive at temperatures ranging from 113º to 160º F. Thermophilic bacteria continue the decomposition process, raising the pile temperature 130º to 160º F, where it usually stabilizes. Unless a pile is constantly fed with new materials and turned at strategic times, the high range temperatures typically last no more than three to five days (Christian *et al.*, 2009). Thermophilic bacteria use up too much of the degradable materials to sustain their population for any length of time. As the thermophilic bacteria decline and the temperature of the pile gradually cools off, the mesophilic bacteria again become dominant. The mesophilic bacteria consume remaining organic material with the help of other organisms.

One can greatly reduce the possibility of pathogens in a pile by excluding pet waste, diseased plants, and manure from diseased animals. Many decomposers are killed or become inactive when pile temperatures rise above 140º F. If the pile temperature exceeds 160º F, you may want to take action and cool the pile by turning it. A number of research projects have shown that soil amended with compost can help fight fungal infestations. If the compost pile temperature goes above 160º F, the composting material may become sterile and lose its disease fighting properties.

2.2.5. USEFUL ACTINOMYCETES

While the various types of bacteria are at work, other microorganisms are also contributing to the degradation process. Actinomycetes, a higher-form bacteria similar to fungi and molds, are responsible for the pleasant earthy smell of compost (Christisn *et al.*, 2009). Grayish in appearance, actinomycetes work in the moderate heat zones of a compost pile. They decompose some of the more resistant materials in the pile such as lignin, cellulose, starches, and proteins.

As they reduce materials, they liberate carbon, nitrogen, and ammonia, making nutrients available for higher plants. Actinomycetes occur in large clusters and become most evident during the later stages of decomposition (Boulter *et al.*, 2002).

2.2.6. USEFUL FUNGI

Like bacteria and actinomycetes, fungi are also responsible for organic matter decay in a compost pile. Fungi are primitive plants that can be either single celled or many celled and filamentous. They lack a photosynthetic pigment. Their main contribution to a compost pile is to break down cellulose and lignin, after faster acting bacteria make inroads on them. They prefer cooler temperatures (70 to 75º F) and easily digested food sources. As a result, they also tend to take over during the final stage of composting (Boulter *et al.*, 2002).

2.3.0. FACTORS AFFECTING COMPOSTING AND AGING PROCESS

There are certain key environmental and physicochemical factors which affect the speed of composting. When these factors are optimised, favourable conditions are provided for microorganisms to produce compost quickly. These factors include, feed stock (C:N ratio), moisture, pH and temperature (Epstein, 1997; Fei-Baffoe, 2010).

2.3.1. FEED STOCK (C: N RATIO)

Organic material provides food for organisms in the form of carbon and nitrogen. Bacteria use carbon for energy and protein to grow and reproduce. Carbon and nitrogen levels vary with each organic material. Carbon-rich materials tend to be dry and brown such as leaves, straw,

and wood chips. Nitrogen materials tend to be wet and green such as fresh grass clippings and food waste (Epstein, 1997). Carbon/nitrogen content can be physically assessed by the fresh, juicy materials that are usually higher in nitrogen and will decompose more quickly than older, drier, and woodier tissues that are high in carbon (Ogunwande *et al*., 2008).

A C:N ratio ranging between 25:1 and 30:1 is the optimum combination for rapid decomposition (Nahm 2003). If ratio is more than 30:1 carbon, heat production drops and decomposition slows (Ogunwande *et al*., 2008). Commonly a pile of leaves or wood chips will stay for a year or more without much apparent decay due to slow decomposition. When there is too much nitrogen, your pile will likely release the excess as smelly ammonia gas. Too much nitrogen can also cause a rise in the pH level which is toxic to some microorganisms (Nahm 2003).

It is difficult to determine an exact C:N ratio without knowing the moisture content of the materials being used. Blending materials to achieve a satisfactory C:N ratio is part of the art of composting. A simple rule of thumb is to develop a volume-based recipe using from one-fourth to one-half high-nitrogen materials (Ogunwande *et al*., 2008).

2.3.2. AERATION

Proper aeration is a key environmental factor need in composting. Composting is an exothermic process often controlled on the basis of temperature feedback, but for which the energetics of the overall system are generally not well known (Ahn *et al*., 2007). Many microorganisms, including aerobic bacteria, need oxygen to produce energy, increase population, and utilize organic carbon to produce compost. Aeration involves the replacement of oxygen deficient air in a compost pile with fresh air containing oxygen (Ahn *et al*., 2007). Natural aeration occurs when air warmed by the composting process rises through the pile, bringing in fresh air from

the surroundings. Aeration can also be affected by wind, moisture content, and porosity (spaces between particles in the compost pile) (Fernandes *et al*., 1994). Porosity can be negatively affected if large quantities of finely sized materials such as poultry droppings, grass clippings, or sawdust are used as heaping reduces air circulation within the pile. However, air circulation can be enhanced if pile is regularly turned. Turning will fluff up the pile and increase its porosity. In poultry litter, such turning is achieved by the rigorous and frequent movement of the birds in the pen, mixing and turning the manure. Another option is to add coarse bedding materials such as leaves, straw, or corn stalks as (Fernandes *et al*., 1994).

2.3.3. MOISTURE

Microbial activity occurs most rapidly in thin water films on the surface of organic materials (Reddy *et al*., 1981). Microorganisms can only utilize organic molecules that are dissolved in water. The optimum moisture content for a compost pile should range from 40 to 60 percent (Winkinson *et al*, 2000). If there is less than 40 percent moisture, bacteria slow down and may become dormant. If there is more than 60 percent, water will force air out of pile pore spaces, suffocating the aerobic bacteria (Himathongkham and Riemann, 1998). Anaerobic bacteria will take over, resulting in unpleasant odours.

The ideal percentage of moisture will depend on the organic material's structure. Straw and corn stalks will need more moisture than leaves, while food waste or grass clippings are not likely to need additional moisture. In a study by Hartel, 2000, survival of fecal coliforms did not increase when the water content of two stacked litter samples were increased by 10% because the moisture content still remained in the range of 40 and 60%. Winkinson, *et al*., 2011, find out that at 35°C, both coliforms and *E. coli* persisted longer under moist (65% wt/wt, wet basis) than dry (30%wt/wt) conditions yet in his conclusion he attributed this observation to temperature. The effects of moisture on coliforms in poultry litter during aging somewhat

remains unclear as different researches have failed to attribute the prevalence of coliforms to moisture.

Since it is difficult to measure moisture, a general rule of thumb is to wet and mix materials so they are about as moist as a wrung-out sponge. Material should feel damp to the touch, with just a drop or two of liquid expelled when squeezed in your hand.

If a compost pile is too dry especially as in the case of poultry litter, it should be watered as the pile is being turned or with a trickling hose. Certain materials such as dead leaves, hay, straw, and sawdust should be gradually moistened until they glisten. These types of materials have a tendency to shed water or adsorb it only on the surface. If a pile is saturated with water, turn it so that materials are restacked. It may also help to add dry, carbon rich material (Reddy *et al.*,1981).

2.3.4. TEMPERATURE

Temperature is another important factor in the composting process and is related to proper air and moisture levels (Reddy *et al.*,1981). As the microorganisms work to decompose the feedstock, they give off heat which in turn increases pile temperatures. Temperatures between 90º and 140ºF indicate rapid decomposition. Lower temperatures signal a slowing in the composting process. High temperatures greater than 140º F reduce the activity of most organisms (Thaxton, 2003).

When organic wastes are gathered into sufficiently large piles for composting, the natural insulating effect of the material leads to a conservation of heat and a marked rise in temperature. The heat given off by the microorganisms further increases the temperature. The temperature

rise inside the pile is due to the difference between the heat generated by the microbes and the heat lost to the surroundings (Christian *et al*., 2009).

The high temperature the pile attains is the most frequently studied mechanism involved in the inactivation of human pathogens during organic waste treatment processes such as composting and deep stacking (Thaxton, 2003). Winkinson *et al*., 2011, studied the effects of heating on aging poultry litter and concluded that high temperatures significantly reduced enteric bacteria after 6 weeks of aging. A similar conclusion was reached by Martin *et al*., 1998 as only 3 out of 64 samples of poultry litter contained quantifiable coliforms after composting. In another study, fresh poultry litter was collected and then composted, fresh litter contained *Escherichia coli* and fecal coliform on Day 1 but not on Day 22 (Haque and Vandepopuliere, 1994). All these studies attributed the decline in microbial numbers to the resulting high temperature (above 60^0C). It is therefore important to measure temperature during the composting to confirm the inactivation of pathogenic microbes. Again the temperature of the compost pile gives an indication of the stage of the compost (Fei-Baffoe, 2010). In Poultry litter composting, however, temperature is hardly, if ever, measured. The decomposition of bedding materials follows a natural process that is not influenced by the farmer and hence poorly monitored. In all the studies described above, poultry litter was monitored under careful condition before high temperatures responsible for pathogenic microbe's inactivation were attained. Concern should therefore be given to the treatment of poultry litter before its reuse as an organic manure to ensure its microbial quality.

2.3.5. OPTIMUM CONDITIONS OF AEROBIC BIODEGRADATION

Below are optimum conditions for aerobic biodegradation to occur (Fei-Baffoe, 2010)

C/N rati0	35 – 50
pH range	5 - 9
Temperature	15 – 55^{0}C
Moisture content	45 – 55%
Porosity (aeration)	25 – 35%

2.4.0. METHODS OF COMPOSTING AND AGING OF POULTRY LITTER

Composting of poultry litter begins after litter has been collected from poultry pen and heaped as means of storing the waste. Therefore, aging is the best term to describe this form of composting (Winkinson *et al.,* 2011). The heap can be turned (turned or open windrow) with time or unturned (static windrow) or composted in Aerobin composter depending on the intended use of the litter (Huang, 2014).

2.4.1. TURNED (OPEN) WINDROW

In this technique, the feed stock (poultry litter) is piled in long rows and turned once or twice per week The turning is intended to provide aeration and release excess heat resulting from degradation (Fei-Baffoe, 2010). Effective turning of the pile also prevents the production of odorous compounds. In areas that receive heavy rainfall, it may be necessary to cover the

windrows so they do not become too wet (Wilkinson *et al.*, 2011). During the active compost period, the size of the windrow decreases with time.

2.4.2. UNTURNED (STATIC) WINDROW

This is similar to open windrow except that the pile is not turned. The composting process takes a longer period if pile is not turned. Unturned windrow requires less labour and supervision. Whether a compost pile should be turned or not depends on the composting materials. It is not advisable to compost materials with high moisture content without turning since it might attract pest.

2.4.3. AEROBIN COMPOSTER

Aerobion uses a patented lung or aeration core inside a sealed bin to promote aerobic break down of organic matter, which experts say is the preferable method to reduce greenhouse gas emissions. The bin is insulalted to prevent heat transfer between the bin and its envrionment. In using the aerobin composter turning of feedstock is not needed as the aeration core ensures effective distribution of air within the bin.

2.5. PATHOGENS ASSOCIATED WITH POULTRY LITTER

Poultry and their manure are well-documented to be main sources of human bacteria pathogens such as *Salmonella* and *Campylobacter* (Beuchat, 2002). Himathongkham, 2000, reported that poultry and their manure are a major source of human foodborne salmonellosis. Preliminary findings of fresh poultry litter from farms in Kumasi indicate a high prevalence of *Salmonella*. Based on these findings, it has been widely suggested that the use of poultry manure as fertilizer in vegetable fields, in particular to crops with short growth period such as lettuce and spring onions, represent a real food safety hazard.

The presence of pathogenic microorganisms are determined by the presence of indicator organisms. Indicator organisms are types of microorganisms used to detect and estimate the level of fecal contamination of a test sample (Georgia Department of Natural Resources**,** 1996). They are not dangerous to human health but are used to indicate the presence of a health risk. In one of many studies of this correlation, when numbers of indicator organisms exceeded 2,000 per 100 mL of sample, the likelihood of bacterial pathogens in the sample was 98.1% (Geldreich, 1970).

World Health Organization Guidelines for Drinking Water Quality state that the presence of this indicator organism, *Escherichia coli*, provides conclusive evidence of recent fecal pollution and should *not* be present in water meant for human consumption. In the U.S.A, the EPA, Total Coliform Rule states that a water system is out of compliance if more than 5 percent of its monthly water samples contain coliforms (USEPA, 1989). These guidelines inform about the close correlation between indicator organisms and pathogenic organisms. Based on the close relationship between indicator organisms and pathogenic organisms, a similar test is used to determine the microbial quality of many of products including poultry manure (Clesceri *et al*., 1998).

Fecal coliforms are another group of indicator organisms, normally inhabiting the intestinal tract of warm-blooded animals; their presence in soil and water indicates that the soil or water was contaminated by fecal material. Thus, these bacteria provide an accepted means of assessing soil and water quality (Clesceri *et al*., 1998). Fecal coliforms consist of several genera of bacteria from the family Enterobacteriaceae that can grow in a selective medium at 44.5 °C for 24 hr.

In this study, indicator organisms including Total Coliform, fecal coliform and *E. coli* were enumerated and used as an indication for the presence of human pathogen in poultry litter.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCE OF POULTRY MANURE

The poultry manure used in this study was obtained from the Bosomtwi District in the Ashanti Region. The district lies within latitude 6º24'N and 6º43'N and longitude 1º15'E and 1º46'W. The vegetation of the district is a deciduous forest and the main occupation of the inhabitants is farming. The district has a unique topography and located within it is Lake Bosomtwi, a natural lake formed by crater.

Rainfall is bimodal with the major season falling between the months of March and July and a minor rainy season around September and October. The dry season is from November to March. Daily minimum and maximum temperatures are 21.20°C and 35.50°C, respectively with a mean temperature of 28ºC (Meteorological Services Department, 2002). Relative humidity ranges between 75 to 79 % with average daily sunshine durations ranging between 2 and 7 hours (Meteorological Services Department, 2002).

More specifically, the poultry manure (poultry droppings and saw dust) samples were collected from Gyasi Poultry farm, a local poultry farm located within the Esreso community in the Bosomtwi District. The farm is in a low-lying area where run-off water collects whenever it rains. The farm area is water logged providing a relatively lower temperature and humidity. The Esreso community has a population of about 4,871 and a growth rate of 2.7% (Ghana Statistical Services, 2002).

Normally, before the poultry droppings are disposed off from the pens, they would have been birds' beddings for at least three months. They are disposed off only when the pen is being prepared to receive new day old chicks.

The Gyasi farm was selected because it is the main source of poultry manure for most vegetable farmers around the Kwame Nkrumah University of Science and Technology (KNUST) and its environs.

Plate 3.1: Preparing manure for transportation to aging site

3.2. SITE FOR AGING

Approximately 500 kg of poultry manure in ten sacks of 50 kg each was collected from Gyasi's farm and transported to the Department of Theoretical and Applied Biology (TAB) - KNUST where the study was conducted.

A shed was constructed to house the manure and to prevent rains from affecting the aging process.

Poultry manure heaps of 3.30 m (width) × 1.50 m (height) × 2.00 m (length) were prepared under the shed in triplicate. A set of three heaps were left unturned throughout the aging process of eight weeks and another set of three was turned using a shovel every 3 to 4 days throughout the eight weeks aging process (Plate 3.2). A third set of poultry manure was stored in a 200kg Aerobin (Plate 3.3).

Plate 3.2: Manure being prepared for aging

Plate 3.3: Poultry manure undergoing aging in Aerobin Composter

3.3.0 ANALYSIS OF SAMPLES

During the aging process, approximately 100 g of poultry manure samples were taken from each of the three set-ups (turned, unturned and Aerobin) and analysed weekly. Samples were collected using gloves and taken from about 0.50 m vertically down into the windrow. The sample was put in sterile bags and sent to the laboratory for analysis of Moisture, pH, temperature and microbial numbers.

3.3.1 MOISTURE

A total of 75 samples were analysed for moisture content using weight loss due to drying (Thiex and Richardson, 2003). Ten (10) grams of samples from each pile was collected in a Petri Dish and subjected to lower drying temperature (105ºC for 3 hours) as recommended by AFIA (2007) using Binder ED23 oven (ED 23, RSA422 Interface). The mass of the petri dish was noted. The samples were then closely monitored for a three (3) hr period after which the final weight (sample and the petri dish) was recorded. Mass of container, wet weight and dry weight were measured using a Sartorius Cubis MSE 10202S top loading electronic balance. Percentage Moisture content was calculated using the formula;

$$\frac{(\text{Wet weight} - \text{Dried weigh}) \times 100\%}{\text{Wet weight.}}$$

3.3.2 TEMPERATURE

Temperature was measured in situ using a Brannan 76 mm immersion thermometer. The tip of the thermometer where the insulation cap is fixed was held when taking reading to avoid the thermometer from taking the reading from the palms of the reader. The reading was recorded only after it stabilised for a minute. Readings were taken from the surface, middle and core of the pile and the average recorded. Surrounding temperature was also recorded simultaneously with pile temperatures.

3.3.3 pH

Ten (10) grams air- dried manure samples were weighed into a 100 ml beaker and 50 ml of distilled water added and the suspension stirred vigorously for 20 minutes and allowed to stand for about 30 minutes by which time most of the suspended particles had settled out from the suspension. The pH was measured using a VRM Phenomenal PH1000H pH meter. The pH meter was calibrated with a blank at pH of 4 and 7 respectively. The electrode of the pH meter was inserted into the partly settled suspension and the pH value read and recorded.

3.4.0 COLIFORM COUNT

Total and Faecal coliforms were estimated using the three-tube Most Probable Number (MPN) method according to standard procedures (Anon, 1998). Ten (10) grams of sample was placed in a stomacher bag and pulsified in 90 ml of 0.1% peptone water for about 45 seconds using a pulsifier (PUL 100E; Stuart Scientific Co. Ltd, U.K). The content of the bag was allowed to settle for about 60seconds after which one milliliter aliquots of the stomacher bag content was used in preparing serial dilutions (10^{-1} to 10^{-7}). One milliliter of each dilution was inoculated

in triplicate into 5 ml of MacConkey broth and incubated for 24 hours at 37°C and 44°C for Total and Faecal coliforms, respectively. All tubes recording colour change from purple to yellow and collection of gas in the Durham tubes were recorded as positive for Total and Faecal coliforms and counts were estimated from MPN tables (Collins *et al*., 1989)

3.4.1. ENUMERATION OF *Escherichia coli*

Escherichia coli was enumerated by transferring 1ml each of the positive tubes for faecal coliforms into 5 ml of tryptophan broth and incubated for 24 hrs at 44°C and then few drops of Kovac's reagent was added. A red ring meniscus indicated the presence of *Escherichia coli* and the MPN table was used for the enumeration.

3.5.0 NUTRIENT ANALYSIS

One hundred (100) grams of the aging manure samples were weighed and oven dried at a temperature 105°C for 3 hours. Each sample was subsequently allowed to cool at room temperature (about 25°C). This enabled maximum removal of moisture in the samples.

3.5.1 Total Nitrogen (N)

Ten (10) grams of air dried soil was weighed into a 500 ml long-necked Kjeldahl flask. Ten millilitres of distilled water was added and allowed to stand for 10 minutes to moisten. One spatula full of Kjeldahl catalyst [mixture of l part Selenium + 10 parts $CuSO_4$ + 100 parts Na_2SO_4] and then 20 ml conc. H_2SO_4 were added. The mixture was allowed to decompose for about 2 hours until clear and colourless or light greenish colour was obtained and the flask was allowed to cool. The fluid was then decanted into a 100 ml volumetric flask and distilled with

NaOH. For the distillation, 10 ml of the aliquot was transferred by means of pipette into the Kjeldahl distillation apparatus provided. Ninety millilitres of distilled water was added to 20 ml of 40% NaOH. The distillate was collected over 10 ml of 4% Boric acid and 5 drops of mixed indicator in a 500 ml conical flask for 4 minutes. The colour change from pink to light blue showed the presence of Nitrogen. One hundred millilitres of the collected distillate was titrated with 0.l N HCl till the blue colour changed to grey and then suddenly flashed to pink.

Percentage Nitrogen was calculated as:

$$\% N = \frac{14 \times (A-B) \times N \times 100}{1000 \times 1}$$

Where,

A = volume of standard HCL used in sample

B = volume of standard HCL used in sample titration

N = Normality of standard HCL

3.5.2 Total Phosphorus (P)

Two grams of manure was weighed into a 50 ml shaking bottle and 20 ml of Bray P1 extracting solution (Extractant) added. This was placed on a mechanical shaker for one minute and then filtered into a 100 ml conical flask. Ten millilitres of filtrate was transferred into a 25 ml volumetric flask with a pipette and then 1.0 ml of molybdate reagent followed by 1.0 ml of the dilute reducing agent added for the solution to develop a blue colour. It was topped up with distilled water to the 25 ml mark, shaken vigorously and the solution allowed to stand for 15

minutes. The percentage transmission at 600 nm wavelength was measured using a spectrophotometer and percentage (%) transmittance (T) recorded.

Calculation:

% T values were converted to 2 – Log T and a graph was plotted using P Standard solutions to obtain actual concentration of P.

The concentration of P in the extract was obtained by comparing the results with a standard curve plotted. From the standard curve, this equation was obtained:

$Y=AX$ …….. (1)

Therefore available phosphorous (P) ppm or mg/Kg

$X=Y/A$ x10

Where

Y= 2- log T of the sample

A = a constant obtained from the graph

3.5.3 Total Potassium (K)

Ten grams of manure was weighed into an extraction bottle and 100 ml of 1.0 NH_4OAc solution added. The bottle with its contents was placed on a mechanical shaker and shaken for 2 hours. The supernatant solution was filtered through No 42 Whatman filter paper and a 10 ml aliquot was taken and potassium content in it determined using a Flame Photometer after calibration with prepared standards.

Calculation:
From the curve, this equation was obtained

Y= BX

Therefore Potassium (K)cmol/kg (X) is calculated as

X=(Y/B) ÷ 39.1

X= Potassium (K)cmol/kg

Y= flame photometer reading of the sample

B= constant value from the curve

Atomic weight of K = 39.10

3.5.4 Organic Matter

A ten gram sample of manure was weighed (W) and put into a porcelain pot and the weight of the sample and pot (W1) noted. It was then put into a furnace for 4 hours at 550^{o}C. The furnace was allowed to cool below 200^{o}C and maintained for 20 minutes and the sample removed and placed in a desiccator with stopper top to cool and then weighed (W2).

Calculations

$$\% \text{ Organic Matter} = \frac{(W1-W2) \times 100}{W}$$

Where,

W1= weight of the sample and crucible
W2= weight of the ash sample and crucible

W= weight of the sample taken

3.6 DATA ANALYSIS

Data collected were analysed by Analysis of Variance (ANOVA) using GenStat software, 6th edition. Mean separation was done using Tukey's Multiple Comparison Test at $P<0.05$. Log transformation of the bacteria count was done using the formula [log10(x+1)], where the value 1 was added to each count (x) in order to eliminate zero data points.

CHAPTER FOUR

4.0 RESULTS

4.1. **Temperature dynamics in Aerobin, Turned and Unturned windrow**

Temperatures increased by 15.05%, 27.90% and 38.71% in the Aerobin, Turned and Unturned windrow, respectively after 14 days of aging (Table 4.2). By week 4, temperatures rose further by 33.33%, 38.71% and 50.54% in Aerobin, Turned and Unturned windrow, respectively.

Temperatures, however, declined from week 5 to week 8 by 27%, 15% and 17% in Aerobin, Turned and Unturned windrow, respectively (Table 4.2).

The Aerobin recorded a mean temperature of 34.6^{o}C (S.E. 1.03), turned windrow 38.1^{o}C and the Unturned windrow 41.7^{o}C (Appendix 1). There were statistically significant differences between temperatures in the Aerobin and Unturned windrow (p= 0.03) after the 8 week period. However, there were no statistically significant differences between temperatures in the Aerobin and turned windrow (p= 0.24) and the turned and unturned windrow (p= 0.10) after the 8 week period (Appendix 2).

Table 4.1: Temperature reading in Aerobin, Turned and Unturned windrow during aging

WEEK/TEMP(^{O}C)	AEROBIN	TURNED	UNTURNED	ATMOSPHERIC
0	31.0	31.0	31.0	30.0
1	32.7	33.3	37.3	30.0
2	35.7	39.7	43.0	32.0
3	36.0	38.0	43.3	33.0
4	41.3	43.0	46.7	33.0
5	36.3	42.7	47.7	33.0
6	32.7	38.3	42.7	32.0
7	33.0	38.7	42.3	33.0
8	33.0	38.3	41.3	32.0

Table 4.2 Percentage change in temperature during aging

WEEK	AEROBIN		TURNED		UNTURNED	
0	31.00	0.00%	31.00	0.00%	31.00	0.00%
2	35.67	15.05%	39.67	27.96%	43.00	38.71%
4	41.33	33.33%	43.00	38.71%	46.67	50.54%
6	32.67	5.38%	38.33	23.66%	42.67	37.63%
8	33.00	6.45%	38.33	23.66%	41.33	33.33%

4.2 pH Dynamics in Aerobin, Turned and Unturned windrow

The pH increased by 13.00% (7.2 to 8.2) in the Aerobin after two weeks of aging but remained at pH 8.2 from week 2 to 6 and then increased again by 2% from week 6 to 8 (8.2 to 8.3) (Table 4.4). In the Turned Windrow, pH increased by 12.50% (7.2 to 8.1) after 4 weeks of aging. However, the pH decreased by 1.39% from week 4 to 6 and then increased by 1.39% by week 8 (Table 4.4). The pH in the Unturned windrow increased by 8.33% (7.2 to 7.8) by week 4 and then decreased by 4.00% (7.8 to 7.5) and remained same till week 8 (Table 4.4).

Composting in the Aerobin recorded the lowest mean pH of 7.6 (S.E. 0.06), followed by the Turned windrow, 7.8 (S.E. 0.10) and the Unturned windrow 8.1 (S.E. 0.20) (Appendix 2).

There was significant difference between Aerobin and Unturned windrow by week 8 ($p= 0.02$). Turned and Unturned was not statistically significant ($p= 0.08$) and Aerobin and Turned was also not also statistically significant ($p= 0.16$)

Table 4.3: pH of manure in Aerobin, Turned and Unturned windrow during aging

WEEK/PH	AEROBIN	TURNED	UNTURNED
0	7.2	7.2	7.2
1	8.2	7.7	7.5
2	8.2	7.9	7.8
3	8.1	7.9	7.7
4	8.2	8.1	7.8
5	8.3	7.9	7.9
6	8.2	8.0	7.5
7	8.3	8.0	7.5
8	8.3	8.1	7.5

Table 4.4 Percentage change (%) in pH during aging

WEEK	AEROBIN	%CHANGE	TURNED	%CHANGE	UNTURNED	%CHANGE
0	7.2	0.00%	7.2	0.00%	7.2	0.00%
2	8.2	13.89%	7.9	9.72%	7.8	8.33%
4	8.2	13.89%	8.1	12.50%	7.8	8.33%
6	8.2	13.89%	8.0	11.11%	7.5	4.17%
8	8.3	15.28%	8.1	12.50%	7.5	4.17%

4.3 Moisture dynamics in Aerobin, Turned and Unturned windrow during aging

Mean moisture content in the aging material was 1.63% in the Aerobin, 2.6% in the Turned windrow and 2.9% in the Unturned windrow (Appendix 4). There were statistically significant differences in moisture content between the Aerobin and Unturned windrow ($p= 0.02$). However there were no statistically significant differences in moisture content between Aerobin and Turned windrow ($p= 0.25$) and the Turned and Unturned windrow ($p=0.06$).

Generally, the moisture content in all the treatments was lower compared to the moisture content of between 45% and 65% needed for microbial activity during composting.

Table 4.5 Moisture content in Aerobin, turned and unturned windrow

WEEK/MOISTURE (%)	AEROBIN	TURNED	UNTURNED
0	0.10	0.10	0.10
1	1.53	2.50	2.84
2	2.01	2.46	2.99
3	1.92	1.46	3.2
4	3.46	2.9	3.2
5	2.1	2.84	3.87
6	1.81	3.36	4.21
7	0.93	4.03	3.62
8	0.86	4.14	2.49

4.4. TOTAL COLIFORM COUNT

Total coliform counts in the aging compost reduced by 93%, 82.25% and 93.12% in the Aerobin, Turned and Unturned windrow, respectively by week 4 (Table 4.7). However, there were slight further decreases in total coliform counts by 3% in the Aerobin, 14.6% in the Turned windrow and 5% in the Unturned windrow from week 4 to 8 (Table 4.7). There were no statistically significant differences between all the treatments.

Table 4.6 Total coliform count in Poultry litter during aging

	Aerobin		Turned		Unturned	
Week	mean count	log count	mean count	log count	mean count	log count
0	741	2.87	741	2.87	741	2.87
2	454	2.66	570	2.76	401	2.60
4	51	1.72	132	2.12	51	1.72
6	23	1.38	23	1.38	9	1.00
8	23	1.38	15	1.20	9	1.00

Count in MPN/g dry weight

Table 4.7 Percentage change in Total coliform numbers during aging

WEEK	AEROBIN	%CHANGE	TURNED	%CHANGE	UNTURNED	%CHANGE
0	741	0.00%	741	0.00%	741	0.00%
2	454	38.69%	570	23.08%	401	45.93%
4	51	93.12%	131.5	82.25%	51	93.12%
6	23	96.90%	23	96.90%	9	98.79%
8	23	96.90%	15	97.98%	9	98.79%

4.5. FAECAL COLIFORM COUNT

Similarly, faecal coliform counts reduced by 68.99%, 22.57% and 83.36% in Aerobin, Turned and Unturned windrow by week 4 (Table 4.9). Faecal coliform counts reduced by more than 95% in all the treatments by week 6 and recorded 97.25%, 95.96% and 95.96% in Aerobin, Turned and Unturned windrow, respectively by week 8 (Table 4.9).

Table 4.8 Faecal coliform count of Poultry litter

	Aerobin		Turned		Unturned	
	mean count	log count	mean count	log count	mean count	log count
0	545	2.74	545	2.74	545	2.74
2	545	2.74	422	2.63	91	1.96
4	169	2.23	454	2.66	10	1.02
6	15	1.20	22	1.35	10	1.02
8	15	1.20	22	1.36	9	1.00

Table 4.9 Percentage Change in faecal coliform during aging

WEEK	AEROBIN	%CHANGE	TURNED	%CHANGE	UNTURNED	%CHANGE
0	545	0.00%	545	0.00%	545	0.00%
2	545	0.00%	454	16.70%	91	83.36%
4	169	68.99%	422	22.57%	10	98.26%
6	15	97.25%	23	95.78%	10	98.26%
8	15	97.25%	22	95.96%	9	98.35%

4.6 *E. Coli* COUNT

E. coli counts reduced by 93.83% in both Aerobin and Turned windrow by week 4 (Table 4.11). In the Unturned windrow, no reduction occurred by week 2, however, 79% reduction was recorded by week 4. At the end of week 8, *E. coli* reductions were 94.93% in Aerobin and 96.70% in both Turned and Unturned windrow (Table 4.11).

Table 4.10. *E. coli* count in Poultry litter

	Aerobin		Turned		Unturned	
week	mean count	log count	mean count	log count	mean count	log count
0	454	2.66	454	2.66	454	2.66
2	454	2.66	422	2.63	454	2.66
4	23	1.38	28	1.46	91	1.96
6	23	1.38	15	1.20	28	1.46
8	23	1.38	15	1.20	15	1.20

Table 4.11 Percentage change in *E.coli* during aging

WEEK	AEROBIN	%CHANGE	TURNED	%CHANGE	UNTURNED	%CHANGE
0	454	0.00%	454	0.00%	454	0.00%
2	454	0.00%	422	7.05%	454	0.00%
4	28	93.83%	28	93.83%	91	79.96%
6	23	94.93%	15	96.70%	23	94.93%
8	23	94.93%	15	96.70%	15	96.70%

4.9. NUTRIENTS ANALYSIS

There were no significant increases in the nutrients (N, P, K, OC) in the manure during the composting process although marginal changes were observed in the amount of nutrients before and after aging (Table 4.12). Unturned windrow recorded the highest nutrient content.

Table 4.12 Nutrients Analysis of poultry litter

	N%	P%	K%	OC%
BEFORE AGING	2.60	2.04	4.80	28.00
AFTER AGING				
AEROBIN	2.70	2.10	5.00	26.00
TTURNED WINDROW	2.60	2.20	4.90	26.00
UNTURNED WINDROW	2.70	2.20	5.10	25.00

CHAPTER FIVE

5.0 DISCUSSION

5.1. VARIATIONS IN TEMPERATURE

This study has shown that although there were temperature increases within the first four week of composting in all the treatments, the ideal recommended composting temperature of above 60^{o}C was not attained. This confirms the work of Christian *et al.* (2009). When optimal initial conditions such as C:N ratio and moisture are set, decomposition of organic matter begins, leading to rise in temperature and a corresponding increase in moisture in a compost pile (Wichuk *et al.,* 2007). However, the limited microbial activity due to the type of feedstock (usable carbon) for this study which does not result in increased heat generation but mostly heat from conservational heat by materials could explain the relatively lower temperatures recorded during the aging compared to active composting (Ahn *et al*., 2007). The marginal rise in temperature observed in the first four weeks is because when organic waste are gathered into sufficiently large piles for composting or aging, the natural insulating effect of the material leads to a conservation of heat resulting in rise in temperature (Christian *et al*., 2009). Again, chemical oxidation that takes place in organic materials such as poultry manure during decomposition results in heat generation however such heat is minimal compared to heat from decomposition (Buggeln and Rynk, 2002). The synergistic effect of these two processes accounts for the marked increase in temperature observed in the study.

The variations in temperature among the treatments as seen in the study could be from the difference in aeration in the different treatments used (Composting Council. 1996). Aeration affects both conservational heat and heat from decomposition (Ahn *et al*., 2007). The unturned windrow recorded the highest mean temperature of 41.7^{o}C because heat lost to the surrounding environment was minimal due to low aeration and the pile could conserve the little heat built up within it. In the case of the turned windrow, heat was lost to the surrounding through the

turning process (Epstein, 1997) which rather increased aeration. According to Epstein (1997), turning incorporates the cooler outer layers of piles into the centre ensuring uniform distribution of heat and heat lost. Turning may not have been favourable in this study as described by Ahn *et al*., (2007) and Christian *et al*., (2009) because high temperatures (above 60º C) have the advantage of killing pathogenic organisms and weed seeds (Composting Council, 1996; Hartel *et al.,* 2000). The Aerobin recorded the lowest temperatures because Aerobins are highly aerated and hence allow a constant exchange of heat with the surrounding environment. However, this property can be fully harnessed in the actual composting process as good aeration prevents the formation of odorous compounds (Epstein, 1997). Without adequate oxygen, the aerobic bacterial population dies off, anaerobic microbes become prevalent, and fermentation occurs (Fernandes *et al*., 1994), leading to production of odorous and other undesirable gases, lower temperatures, a slower decomposition rate and low pH (Alexander, 1995). On the other hand excessive aeration can keep a pile too cool for optimum microbial activity (Christian et al., 2009).

Therefore, the different level of gas permeability (aeration) in the treatments possibly accounts for the variation in temperature between the treatments observed in the study.

5.2. VARIATIONS IN pH pH of a compost pile is most often used as a diagnostic tool (Alexander, 1995). Recorded pH during the aging process for all the treatments was within the

optimal pH range for composting (5- 9) (Fei-Baffoe, 2010). Though the pH was within the generic range, most were in the alkali range. Such high pH informs that little or no decomposition is taking place in the pile as lower pH during composting is as a result of organic acids produced as by-products of the degradation process (Haapapuro *et al.,* 1997). Low pH (between 3-6) informs the farmer that anaerobic condition are persisting and decomposition is slow (Alexander, 1995; Gresham *et al*., 1992). On the contrary, high pH as observed in this study promotes production of ammonia in the pile (Gresham *et al*., 1992).

If pH within the alkali range persist in a compost pile, it is most often used as a diagnostic tool to suggest slow degradation (Composting Council, 1996). Therefore having alkali pH range as observed in this study is an indication that favourable conditions needed for decomposing microorganism to act was not established in the feedstock used in the study (Alexander, 1995).

5.3. MOISTURE CONTENT DURING AGING OF POULTRY LITTER

Throughout the study, moisture content in the composting material irrespective of the treatment were all below 10%; an indication that the poultry litter is very dry (Himathongkham and Riemann, 1998). It is important to maintain a moisture content of 45 to 65 percent throughout the entire composting as too much water fills up the air spaces, which creates undesirable anaerobic (oxygen limiting) conditions and too dry materials cause desiccation of microbes (Gresham *et al*., 1992). The very low moisture content recorded, resulted in reduced microbial activity as discussed by Wilkinson *et al.,* (2011). In turn, other factors that are dependent on microbial degradation such as temperature and pH and the moisture content were affected (Pare *et al*., 1998) as water released during decomposition increase moisture content, creating enabling environment for decomposing bacteria to act (Alexander, 1995). The low moisture content therefore explains the decreased activity of the decomposers (microorganisms) in the

piles resulting in minimal degradation and onward lower temperature during the aging (Bernal *et al*., 1998; Pare *et al*., 1998).

According to Bernal *et al*. (1998) and Pare *et al*. (1998) on rewetting a dry compost pile, an ideal environment is provided for microorganisms to repopulate. However in this study rewetting was not done indicating that both decomposing bacteria and pathogenic were presented with a hostile environment which affected their population (Soares *et al*., 1995).

Among the treatments used, Unturned windrow recorded the highest moisture content of 4.21% at week 6. This could be due to minimal microbial activity and low gas penetration that occurred as the manure aged (Wilkinson *et al.*, 2000). On the basis of aeration, it could be then explained why Aerobins recorded the lowest moisture content. Aerobins are well aerated and that may have led to desiccation in the material (Fernandes *et al*., 1994).

Such low moisture of less than 10% could be effective in controlling the population of pathogenic microorganisms during aging but may as well limit the activities of decomposing bacteria

5.4 TOTAL AND FECAL COLIFORMS

Factors that govern the persistence and growth of bacteria in compost materials such as poultry manure are complex including, animal health, manure management, seasonal variations and aging (Composting Council, 1996). Coliforms persisted in the litter even after the active degradation phase (composting). However, the numbers reduced with increased age of the litter confirming work done by Hartel *et al*., (2000). Coliform numbers reduced by more than 90% after eight (8) weeks of aging in all the treatments. The findings made in this study is in agreement with earlier workdone by Wilkinson *et al*., (2011) suggesting that aging affects microbial quality of poultry manure.

The survival of coliforms in poultry litter is mostly affected by temperature, moisture, pH, nutrient supply and solar radiation (Crane *et al*., 1986; Thaxton, 2003). However, because the windrows were covered and kept under sheds, there could be reduced effect of direct sunlight and solar radiation. Similarly, pH range of 5.8 to 8.4 do not adversely affect coliform numbers (Lambert, 1974). High temperatures therefore may be the most likely parameter that resulted in the inactivation of the pathogens although they were not above 60^{o}C as observed in this study (Thaxton, 2003). Such high temperatures needed for pathogen inactivation could still be achieved during aging despite low rates of microbial decomposition, probably because the litter had low thermal conductivity and low gas permeability (Wilkinson *et al*., 2011). Again chemical oxidation that takes place during decomposition of organic matter could be an additional cause of increased temperatures in the manure, which in some cases leads to spontaneous combustion in dry conditions (Buggeln and Rynk, 2002). Other contributors to the microbial die off could be moisture, nutrient supply and microbial antagonism (Fernandes *et al*., 1994; Himathongkham and Riemann, 1998).

Water is needed to hydrolyse nutrients for microorganism to use (Reddy *et al*.,2001). As litter remained very dry throughout the study, it was a hostile environment for the persistence of coliforms (Wilkinson, 2011).

Inadequately nutrient supply also contributed to the death of coliforms as the litter aged. Coliforms and other microorganisms present during aging had to compete over the residual usable carbon. Again coliforms were not able to reduce their metabolic rate to meet the low availability of usable organic carbon (Sadik *et al*., 2012). The limited availability of usable carbon was as a result of degrading microorganism using up such carbon during the active degradation phase (Soares *et al*., 1995).

Unturned windrow was the most efficient treatment that resulted in highest reduction of coliforms (98.79% after six weeks) though all the two others were effective. This was because

a corresponding high temperature and low moisture were observed in the Unturned windrow. These factors coupled with low aeration in the Unturned windrow created a stressful environment for the survival coliforms (Sadik *et al*., 2012). Reduction of coliform followed a pattern where treatments that recorded higher temperatures had lower counts. Aerobin < Turned windrow < Unturned windrow.

Aging of poultry manure before its final application to vegetable farm lands was effective in controlling the population of coliforms. Vegetable farmers are therefore advised to age manure using Unturned Windrow method for at least four weeks before onward application.

5.5. NUTRIENT ANALYSIS

Nitrogen, Potassium, and Phosphorus marginally increased as the litter aged. The marginal increases observed may be as a result of relatively lower mineralization that took place during the aging (Soares *et al.*, 1995). Because, conditions such as moisture and C:N were not optimal in the feedstock used, biodegradation of organic matter to produce inorganic nutrients could not take place rapidly as it occurs during active composting (Fernandes *et al*., 1994). However chemical oxidation that takes place in organic materials such as poultry manure when organised into piles (Buggeln and Rynk, 2002) may have worked in synergy with minimal biodegradation to marginally increase the content of nitrogen and potassium (On farm composting handbook, 1992); accounting for the relatively small increases observed

Aging did not effectively increase nutrients in all the treatments used, however, aging improved the microbial quality of the manure. To improve on nutrient, poultry manure can be co composted with leaves during aging or curing stage (Composting Council, 1996)

CHAPTER FIVE

6.0. CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The outcome of this research as seen in the data presented provides evidence that specific onfarm management practices such as aging influences the prevalence of pathogens in poultry manure.

It was observed in the study that aging enhanced temperature increase and maintained optimal pH for the reduction of pathogenic microorganism.

Simple on-farm management practice such as aging of manure for at least six weeks therefore, reduces the risk of microbial contamination on both fresh produce and manure handlers such as farmers. Again it assures consumers of the microbial quality of vegetables produced with poultry manure as organic fertilizer.

Aging did not affect fertilizer value significantly in this study however some marginal increases in macro nutrients were observed.

Keeping poultry manure in unturned heaps and covering the heaps to keep the manure dry for a minimum of six weeks before applying to farmlands was the best treatment in improving the microbiological quality of the manure.

6.2 RECOMMENDATIONS

Based on the outcome of the study, the following recommendation are suggested:

1. Vegetable farmers who use Poultry manure should consider heaping or stacking poultry manure for a reasonable period of time (minimum of 6 weeks) to eliminate coliforms so as to prevent contamination for water bodies by runoff from land spread poultry manure.
2. During aging of poultry manure, conscious efforts should be taken to keep the manure dry throughout the aging period as survival of coliforms was significantly affected by low moisture.
3. When poultry manure collected for aging is still undergoing active decomposition, it is advisable to periodically turn it to ensure uniform distribution of temperature within the pile and to prevent anaerobic conditions. In such situation the use of Aerobin composter is highly recommended.

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APPENDICES

Appendix 1 One-way analysis of variance of temperature

P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
F	12.33
R squared	0.5361
Bartlett's test for equal variances	
Bartlett's statistic (corrected)	14.24
P value	0.0026
P value summary	**
Do the variances differ signif. (P < 0.05)	Yes

Appendix 2 Turkey's multiple comparison test of Temperature recorded

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
Aerobin vs Turned	-3.478	2.924	No	-8.038 to 1.082
Aerobin vs Unturned	-7.067	5.942	Yes	-11.63 to -2.507
Aerobin vs Atmospheric	2.522	2.121	No	-2.038 to 7.082
Turned vs Unturned	-3.589	3.018	No	-8.149 to 0.9712
Turned vs Atmospheric	6	5.045	Yes	1.440 to 10.56
Unturned vs Atmospheric	9.589	8.062	Yes	5.029 to 14.15

Appendix 3 Statistical Analysis of pH

One-way analysis of variance		
P value		0.002
P value summary	**	
Are means signif. different? (P < 0.05)	Yes	

Number of groups	3
F	8.17
R squared	0.4051

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	2.528
P value	0.2825
P value summary	ns
Do the variances differ signif. (P < 0.05)	No

Appendix 4

Tukey's multiple comparison of pH

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
Aerobin vs Turned	-0.2889	3.093	No	-0.6188 to 0.04101
Aerobin vs Unturned	-0.5333	5.71	Yes	-0.8632 to -0.2034
Turned vs Unturned	-0.2444	2.617	No	-0.5743 to 0.08546

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	95% CI of diff
Aerobin vs Turned	-1.008	2.664	No	-2.019 to 0.003287
Aerobin vs Unturned	-1.311	3.466	Yes	-2.322 to -0.3000

Turned vs Unturned	-0.3033	0.8019	No	-1.314 to 0.7077

Appendix 5 Statistical Analysis for moisture

Repeated Measures ANOVA	
P value	0.0082
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	3
F	6.586
R squared	0.4515

Was the pairing significantly effective?	
R squared	0.5282
F	4.082
P value	0.0081
P value summary	**
Is there significant matching? (P < 0.05)	Yes

Appendix 6 Bonferroni's Multiple Comparison test of moisture

Appendix 7 Statistical Analysis of Total coliform

One-way analysis of variance	
P value	0.9089
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	0.09652
R squared	0.021

ANOVA Table	SS	df	MS
Treatment (between columns)	0.1106	2	0.0553
Residual (within columns)	5.156	9	0.5729
Total	5.267	11	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Aerobin vs Turned	-0.125	0.2335	No	ns	-1.695 to 1.445

Aerobin vs Unturned	0.11	0.2055	No	ns	-1.460 to 1.680
Turned vs Unturned	0.235	0.4391	No	ns	-1.335 to 1.805

Appendix 8

Statistical Analysis of *E. coli*

One-way analysis of variance

P value	0.9137
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	0.09115
R squared	0.01985

ANOVA Table	SS	df	MS
Treatment (between columns)	0.08972	2	0.04486
Residual (within columns)	4.429	9	0.4922
Total	4.519	11	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Aerobin vs Turned	0.0325	0.06552	No	ns	-1.423 to 1.488
Aerobin vs Unturned	-0.165	0.3326	No	ns	-1.620 to 1.290

-1.653 to					
Turned vs Unturned	-0.1975	0.3981	No	ns	1.258

One-way analysis of variance of *E.coli*

P value	0.9996
P value summary	Ns
Are means signif. different? (P < 0.05)	No
Number of groups	3
F	0.0003743
R squared	0.00008317

ANOVA Table	SS	df	MS
Treatment (between columns)	0.095	2	0.0475
Residual (within columns)	1142	9	126.9
Total	1142	11	

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
-23.34 to					
aerobin vs turned windrow	0.025	0.003138	No	ns	23.39
-23.17 to					
aerobin vs unturned windrow	0.2	0.02511	No	ns	23.57
turned windrow vs unturned windrow	0.175	0.02197	No	ns	-23.19 to 23.54