ISOLATION CHARACTERISATION AND MOLECULAR STUDIES OF SOME PATHOGENIC BACTERIA IN COMPOSTING ANIMAL AND MUNICIPAL WASTES IN ZARIA, KADUNA STATE, NIGERIA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY OF SCEINCE IN MICROBIOLOGY

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DECLARATION

I declare that the work in this Dissertation entitled "Isolation, Characterisation and Molecular Studies of some Pathogenic Bacteria in Composting Animal and Municipal Wastes in Zaria, Kaduna State, Nigeria" has been carried out by me in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma in this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This Dissertation entitled "ISOLATION, CHARACTERISATION AND MOLECULAR STUDIES OF SOME PATHOGENIC BACTERIA IN COMPOSTING ANIMAL AND MUNICIPAL WASTES IN ZARIA, KADUNA STATE, NIGERIA" by Ocholi YAHAYA meets the regulations governing the award of the degree of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to my Late Mother Mariam Yahaya. May her soul rest in Allah's *aljenetul firdos*

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ABSTRACT

Composting of raw organic waste is an excellent example of high biological processes used for agricultural practices. The open pile and windrow method were used in composting poultry, cow, and municipal solid waste for twelve (12) weeks. Temperatures, pH and some selected heavy metals were determined during composting and in the finished products. The presence of helminths was determined and the total plate count, total coliform counts, the antibiotic sensitivity patterns of *Salmonella typhi, Vibrio cholerae* and *E.coli 0157:H7* and their molecular profiles were also studied. All the heavy metals in the final compost was lower than the standard recommended by the USEPA, the temperature conditions did not meet the 55^{o}C recommended for compost throughout the 12 weeks of composting . All the helminths did not survive beyond two weeks of composting. The results also showed a steady decline in total plate and coliform counts, and showed no significant difference between both. Composting techniques did not affect microbial growth but were significantly affected by the duration of composting. A total of 109 *Vibrio*, 152 *E.coli*, and 105 *Salmonella* isolates were tested using biochemical and serological tests. Out of these number 97(88.9%) *Vibrio* isolates were confirmed to be *Vibrio cholerae*, 140 (92.1%) were *E.coli* 0157 and 98 (93.3%) were *Salmonella typhi.* From these positive cases, 45 (46.4%) *Vibrio cholerae*, 64 (45.7%) *E.coli* 0157 and 47(48%) *Salmonella typhi* were from the open pile methods while 30 (30.9%) *Vibrio cholerae*, 76(54.3%) *E.coli* 0157 and 51(52%) *Salmonella typhi* were from the windrow method of composting. The organisms were evaluated by DNA based method using the 16S rDNA of the microbial community to verify and complement molecular and serological identification procedures. 16S rRNA genes fragments were amplified using

universal primers and sequenced. The antibiotic susceptibility pattern showed higher resistance among the isolates from poultry droppings and cow dung. and the overall quality of the final compost met the recommended standard by the European Union for finished compost. Large scale, wide spread and further researches are highly recommended.

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CHAPTER ONE

1.0 INTRODUCTION

Composting is an excellent example of a highly complex biological process, involving many species of bacteria and fungi which convert a low-value material into a higher value product. A wide range of biological wastes can be composted including materials generated by agriculture, food processing, wood processing, sewage treatment, industrial, municipal waste and animal wastes.

Composting is a process of controlled biological decomposition of biodegradable materials under managed conditions that are predominantly aerobic and allow the development of thermophilic temperatures as a result of heat produced biologically, in order to achieve compost that is stable and less harmful when used. The agricultural or domestic use of compost may possibly increase the risk of disease transmission by a number of mechanisms. The most obvious is direct contact by humans handling the material or animals grazing pastures or crops on which the material has been used as a organic fertilizer. This could be particularly important for organisms such as enterohaemorrhagic *Escherichia coli O157:H7* which is thought to have a low infectious dose (possibly as few cells may initiate an infection in susceptible humans) (Tarr, 1995; Anon, 1997; Ritu, *et al.,* 2011).

The microbial community (biomass) was largest in compost at 40^{o}C, and substantial differences were seen (Wichuk and McCartney, 2007). The development of markets for compost products remains a key challenge to the promotion of a greater uptake of composting. Many countries which have encouraged composting strongly have put in place a statutory standard supported by systems for quality assurance. The publication of the Publicly Available Specification (PAS) 100 for composted materials in 2002 was an important step towards a British Standard for compost (BSI, 2002). Composting relies upon indigenous population of microorganisms from the environment carried by most organic materials and are generally inefficient in trapping energy released during the oxidation of organic substrates. Energy that is not biochemically captured in the catabolic degradation of substrates is dissipated to the environment as heat. This is not normally noticed when the material is spread over a large area of ground. But compost piles restrict the dissipation of heat, leading to an increase in temperature (Zibilske, 1998).

Microbial populations change during the process through the mesophilic stage (20-40^{o}C), thermophilic stage (>40^{o}C), stabilization or curing stage (cooling period) (Jones, 1976). Organisms commonly encountered during composting are said to include *Salmonella sp, Shigella sp, Yersinia sp, Mycobacterium tuberculosis, Vibrio cholerae*, *E. coli* (and particularly enteropathogenic [EPEC], enterohaemorrhagic [EHEC] and enterotoxigenic [ETEC] strains), *Campylobacter jejuni*, *Listeria monocytogenes*, *Yersinia enterocolitica, Shigella dysenteriae, Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Clostridium botulinum, hepatitis viruses, Norwalk virus*, *Arboviruses* and *Caliciviruses* (Cook, 1991). Others include *Ascaris lumbricoides*, *Cryptosporidium*, *Giardia spp, Entamoeba histolyica* and *Endolimax nana,* and composting has proved to be an effective means of reducing these pathogens to a non detectable level (Gibbs *et al.*, 1998).

1.2 Statement of the Problem

In spite of the wide variations among the limit values and the number of tested parameters required by compost organizations, all national compost standards have not been given special considerations. The compost properties associated with public health and environment contaminants include heavy metals, pathogens and potentially toxic organic compounds (Brinton, 2000; Hogg *et al.*, 2002).

Manures are generally packed in bags and sold to farmers for land application. Some big farms also manufacture compost by primitive methods and sell the product in unlabeled bags and are used widely by farmers as plant fertilizer. Recently, many African countries have not established laws governing the production and sale of organic fertilizers, which should propose some specifications and regulations for compost quality (GCST, 2006). In Nigeria, there are neither mechanisms nor organizations responsible for the evaluation of compost, which may result in low quality that is harmful to public heath, plant and the environment. There is paucity of information on maturity and stability indices of local composts.

1.3 Justification

Animal and human wastes are used for agricultural purposes in many parts of Nigeria and this increases the risk of disease transmission. The most obvious is the direct contact by humans handling the materials or animal grazing pasture or crops on which the materials had been used as fertilizers. Nigeria has a high population density with relatively poor infrastructure and available sanitary facilities cannot sustain population leading to waste being scattered all around. Most of the wastes generated are from organic sources and could be washed into wells as run offs. Many diseases out breaks had been reported as a result of this and significant risks to human life may occur from exposure to pathogens in well water from which drinking water is derived (Adeyeba and Akinbo, 2003).

Land application of composted animal manures can be one of the most economical and attractive methods to solve two problems: waste disposal and the necessity to increase the organic matter content of soil (Giusquani *et a*l., 1988; Changa *et al*., 2003; Bernal *et al.*, 2008). Composts had been shown to enhance soil fertility and quality and to improve crop productivity, but application of unstable or immature composts may have adverse effect on plant growth, environment and public health (Bernal *et al.*, 1998; Chukwujindu *et al*., 2006; Bernal *et al.,* 2008). Several official and private organizations in different countries have established standards and specifications for compost quality to improve crop production and to protect public health and environment (De Bertoldi, 1993; AS99, 1999; Brinton, 2000). Examples of fairly developed compost standards are those produced by California Compost Quality Council (CCQC, 2002) and British Standards, PAS-100-2005 (BSI, 2005).

Proper understanding of the quality profiles of compost and their safety determination will not only be significant but also generate the need for the development of simple technique that can be handled by everyone to generate compost whose quality profiles comply with international standards.

1.4 Aim:

The aim of this study is to isolate and characterize surviving pathogenic bacteria during composting waste.

1.5 Specific Objectives

The objectives of this study are as follows,

1. To determine the physicochemical parameters of the various wastes samples

2. To isolate and characterize the target pathogens (*E.coli* 0157:H7, *Vibrio cholerae* and *Salmonella typhi*).

3. To isolate the helminthes eggs and larvae in the sample.

4. To determine the antibiotic susceptibility pattern of the bacterial isolates

5. To determine the molecular characteristics of the bacterial isolates

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Composting

Composting transforms organic matter into a stable product of humus-like substance. The end product can be used for agricultural purposes. Composted raw matter is of various origin including yard waste, manure and sewage sludge (SS) and municipal solid waste (MSW). Microbial contamination of waste is mainly of faecal origin, especially for SS or MSW. It is known that soil is a recipient of solid waste which contains enteric pathogens in high concentrations. Gastrointestinal infections are the most common disease caused by enteric bacteria. Some examples are Salmonellosis, Vibriosis, Shigellosis and other infections caused by *Campylobacter jejuni*, *Yersinia sp and E.coli 0157:H7* and many other strains.

Viruses are the most hazardous and have some of the lowest infectious doses of any of the enteric pathogens. Hepatitis A, Hepatitis E, Enteric Adenoviruses, and Poliovirus type 1 and 2, multiple strains of Echoviruses and Coxsackievirus are associated with waste (Santamaria and Toranzos, 2003). Wichuk and McCartney (2007) advised that since the infective dose for many of these organisms is very low, it is generally accepted that pathogens should be reduced to non-detectable levels, and that compost should be maintain at 55^{o}C for at least three days.

However, survival of pathogenic bacteria, protozoa and helminthes occurred in a significant number of studies, despite the prescribed time –temperature conditions apparently being met. For example, Strom (1985) reported that up to fifteen (15) taxas were isolated, including 10 genera, which dominated most samples after composting, the plague causing *Yersinia pestis* was consistently found in static pile compost and a significant increase in bacterial population including *Salmonella, Vibrio, and* helminthes in compost was also reported (Turner *et al*., 2005). It has also been reported that *Campylobacter* in cattle manure can survive composting and persist for a long period in the final product (Inglis *et al*., 2010).

The microbial community (biomass) was largest in the run at 40^{o}C, where substantial differences were seen in the microbial community structure and the succession compared to thermophilic temperature (Alam *et al*., 2006; Yanko, 2008). Although the use of sludge in soil amendment is attractive, it is not without potential health risks, toxic chemicals, including heavy metals and industrial organic waste may enter the food chain and present long-term health risks. Francis *et al.* (2003) reported that the number of coliform count in compost manure from feed lot pen decline as the composting progresses, and that *E.coli* was eliminated in the first 7 days when the average windrow temperature range from 33.5^{o}C to 41.5^{o}C. The type bedding did not influence the total plate count or *E.coli* count. However, the total aerobic heterotroph populations remain high (>7.0 log 10 cfu dry weights). Schleiff and Dorn (1997) reported that *E.coli* would be cultured from dry poultry (*Gallus gallus domesticus*) manure after 88 days of composting. Cointrau (2003) reported that the number of fecal streptococci, fecal coliform bacteria and clostridia count were significant, although counts declined with composting.

The quality assessment of commercially produced compost in Saudi Arabia market showed that compost samples were contaminated with feacal coliform, *Salmonella* and *Staphylococcus sp* respectively, at levels above the suggested limit proposed by regulatory agencies (Al-Turki, 2010). The agricultural or domestic use of compost may possibly increase the risk of disease transmission by a number of mechanisms. The most obvious is direct contact by humans handling the material or animals grazing pastures or crops on which the material has been used as a fertilizer. This could be particularly important for organisms such as enterohaemorrhagic *Escherichia coli O157:H7* which is thought to have a low infectious dose (possibly as few as 10 cells may initiate an infection in susceptible humans) (Tarr, 1995; Anon, 1997). Indeed, direct contact with ruminant faeces on farms and on contaminated pastures has resulted in outbreaks of *E. coli O157:H7* (O'Brien *et al.,* 2001; Ogden *et al.,* 2002; Chukwujindu *et al*., 2006).

The occurrence and survival of pathogens in human sewage and animal wastes and the subsequent survival and infectivity of organisms when these materials are disposed of on land has been extensively reviewed (Ellis and McCalla, 1978; Strauch, 1978; Jones, *et al*.,1980a; Jones *et al*., 1980b; Jones *et al.,* 1980; Jones, 1981; Kent *et al.,* 1981; Jones, 1982; Jones, 1984; Jones, 1986; Jones, 1992; Jones and Martin, 2003, Strauch and Ballineri, 1994; Strauch, 1996; Dumontet *et al.*, 1999).

Jones (1982) described the pathogens most commonly found in animal wastes which could be transmitted to other animals. This list could be extensively updated, but the main risk organisms are still *Salmonella,* pathogenic *E. coli*, *M. tuberculosis* and *M. bovis*. In agricultural use of animal wastes these organisms can be controlled by codes of practice limiting spreading and grazing by animals.

Pell (1997) indicated that there are numerous pathogens in livestock manure which can infect humans. The principal ones are *Cryptosporidium parvum, Giardia sp., Listeria. monocytogenes, E. coli O157:H7, Salmonella sp.* and *M. paratuberculosis.* The type of pathogens most commonly found in sewage and sewage sludge depends on the state of health of the population, as well as the presence of hospitals, meat processing plants and abattoirs in the area (Jones *et al.,* 1980a; Jones *et al.,* 1980b; Bruce and Davis, 1983).

Organisms commonly encountered include *Salmonella sp* and *E. coli*, but a number of others including *Streptococcus, Campylobacter sp* and Mycobacteria may be present usually at a lower concentration. Epstein (2002) reported on the occurrence of pathogens in biosolids, municipal solids waste, food waste, wood waste and yard waste. Organisms reported included *Salmonella, Shigella, Yersinia spp, M. tuberculosis, Vibrio cholerae, E. coli, Listeria sp* and a number of viruses, protozoa and Helminthes. Many are rare or do not occur in the UK. Typically, temperatures reached in a well-managed compost operation are in a range of 50 to 65^{o}C. Such temperatures are well above the thermal death points of mesophilic pathogens (Golueke, 1982). As the temperature of the composting process increases, pathogens are usually destroyed as they reach their thermal death points. The survival of bacteria is variable but most viruses are killed in about 20 minutes at 70°C (Day and Shaw, 2000). There is a relationship between temperature and time. A high temperature for a short period or a lower temperature for a longer period may be equally effective. Epstein (2001) reported that high temperatures were extremely effective in the destruction of pathogens.

2.2 Indicator Organisms, Pathogenic *E. coli and Salmonella.*

Pereiro- Neto *et al*. (1986) studied the survival of indicator organisms in sewage sludge composted with solid municipal wastes. The organisms used to monitor the effectiveness of the composting process to produce a sanitized waste were *E. coli,* faecal streptococci and *Salmonella sp.* They concluded that static aerated piles were more efficient than windrows in the inactivation of the indicator organisms. In static aerated piles, *E. coli* was reduced below the detection level, faecal streptococci were reduced to less than 10^2 cfu per gram and *Salmonella spp* were completely eliminated after 32 days of composting. In contrast, all of these organisms were still detectable at the end of the windrow composting process. Hays (1996) however, reported that in a well-managed biosolids windrow composting operation indicator organisms and pathogens including *E.coli* and *Salmonella sp* were destroyed and Gaby (1975) found that *Salmonella* and *Shigella spp* disappeared in windrow composting of municipal solid wastes in 7 to 21 days.

The survival of *E. coli O157:H7* in green compost has not been reported. Maule (1998) reported that *E. coli O157:H7* survived for 56 days in fresh cattle faeces and up to 9 days in cattle slurry at 18 ^{o}C, while Kudva *et al*. (1998) were able to isolate this organism from a manure stack for as long as 21 months. Similarly, *E. coli O157:H7* appears to survive for long periods in bovine manure mixed with bedding. Wang *et al.* (1996) reported the survival of an initial inoculum of 10^5 cfu per gram of this organism for 70, 56 and 49 days respectively at 5, 22 and 37°C. Kudva *et al*. (1998) demonstrated that *E. coli O157:H7* survived in a manure pile from sheep for 21 months. In a pile which was aerated the organism survived for 4 months. In bovine manure the survival time was 47 days. Once the manures were aerated and heated survival times were reduced such that at temperatures above 45^{o}C the organism was not detected after 2 days. Himathongkham *et al.* (1999) reported that this organism declined exponentially in cattle manure with a decimal reduction time (DRT) of 6 days to 3 weeks and in cattle slurry with a DRT of 2 days to 5 weeks. The most rapid destruction was achieved at 37^{o}C and numbers of *E. coli* declined at approximately the same rate as *S. typhimurium*. In poultry manure the concentration of *E. coli O157:H7* and *S. typhimurium* also declined exponentially. The DRT ranged from 12 hours to 1-2 weeks at 4°C (Himathongkham *et al.*, 2000).

Survival for shorter periods in composted wastes should be predicted, although Droffner and Brinton (1995) reported the survival of *Salmonella* and *E. coli* in industrial composts for up to 59 days at 60^{o}C. This study relied upon the use of gene probes rather than isolation of viable organisms, which may account for the extended survival period reported. In contrast, Turner (2002) found that an indicator strain of *E. coli* was inactivated in farmyard manure, pig faeces and straw if kept at 55^{o}C for more than two hours. It was, however, still viable after 72 hours at 50^{o}C. Coliform grew in the compost if the process was carried out at mesophilic (37^{o}C) temperatures. Knoll (1961) described several experiments where *Salmonella* strains were subjected to different composting temperatures. After 14 days at 55 to 60°C the final compost did not contain salmonellas. Thermal destruction of bacteria may depend on factors other than temperature, including moisture content, ammonia concentration and the presence of other organisms.

In industrial compost *Salmonella* and *E. coli* were found to survive for 59 days at 60^{o}C (Droffner and Brinton, 1995). Lung *et al.* (2001) inoculated *E. coli O157:H7* and *Salmonella enteritidis* into a bench-scale cow manure composting system at a level of 10^{7} organisms per gram of raw compost. *E. coli* was not detected after 48 hours at 45^{o}C and *S. enteritidis* after 72 hours. When the compost was held at 25^{o}C the concentration of seeded organisms did not decline. The effect of temperature was also demonstrated by Gibbs *et al.* (1998). They investigated the effect of two windrow composting systems on the survival of

Salmonella spp which was not detected after composting at 52 to 53°C but was isolated from 7 of 11 samples at 30 to 40°C. Tiquia *et al.* (1998) found that *Salmonella sp* was no longer detected after composting for 3 weeks in a mixture of partially decomposted pig manure and sawdust at temperatures between 64 and 67^{o}C. Hirn *et al.* (1983), using compost derived from sewage sludge and food waste, did not isolate *Salmonella sp*. Faecal streptococci and *C. perfringens* were always present, although numbers declined by 2 to 3 logs during a 6- to 7-week period. Plym-Forshell (1983) studied the survival of Salmonellae in cattle and pig manure mixed with straw. *S. dublin*, *S. senftenberg* and *S. typhimurium* survived for less than 7 days in cattle manure at 55 to 60°C or pig manure at 54 to 60°C (Wong, 2002a). In contrast, *S. derby* persisted for more than 140 days at 2 to 19°C.

Russ and Yanko (1981) studied the kinetics of development of sludge compost inoculated with *Salmonella sp* under aerobic and anaerobic conditions. During the anaerobic incubation *Salmonella spp* were detected until 32 day of incubation, while during the aerobic incubation *Salmonella spp* decreased to an undetectable level in about 15 days. Burge *et al.* (1978) reported that *Salmonella spp* were destroyed in 10 days by aerated, static pile composting of raw sludge in 15 days in turned windrows. Similarly, Knoll (1961) described several experiments in which *Salmonella* strains were subjected to composting at a biosolids refuse plant. The *Salmonella spp* were eliminated after 14 days at 55 to 60°C. At a higher temperature of 60 to 70°C, *S. newport* could not be isolated from composted sewage after 3 days (Wiley and Westerberg, 1969). At a similar temperature (60 to 65°C) *S. typhimurium* and *Serratia marcescens* inoculated into a composting drum containing septic waste, biosolids and municipal solid wastes could also not be isolated after 3 days (Krogstad and Gudding, 1975). Swain *et al*. (2006) reported that *Phytopthora vamorum* were not recovered at the end of composting process regardless of the isolation technique used.

The extensive recent study on the survival of indicator organisms (*E. coli* and *Enterococcus faecalis*) and *Salmonella sp* during composting in 4 facilities in Denmark, Sweden, Norway and Finland revealed that sewage sludge mixed with straw yard waste and straw was composted in open-air windrows without cover or forced aeration for 7 weeks followed by stabilization for 2-3 months in Denmark. In Sweden, source-separated household waste mixed with yard waste was composted in force-aerated boxes with semi-permeable covers; after a sanitation phase of 3 weeks the compost was moved to a force-aerated box without a roof for a 4-week stabilization phase followed by a second stabilization phase in open-air windrows for an additional 4 months. In Norway, source-separated household waste was mixed with yard and forestry waste and composted in open-air windrows without cover or forced aeration; windrows were combined after 6 weeks and left to stabilize for 6 weeks then 2 to 3 months.

In Finland, sewage sludge mixed with wood chips and peat was composted in fully-enclosed, aerated tunnels for 7 days, followed by a second sanitization period of 7 days in a second tunnel and a stabilization phase of 6 months in an open-air windrow. During composting variations in temperature at different levels in the compost occurred at all 4 facilities. Mean temperatures were, respectively, 50-66°C, 45-74°C, 62-66°C and 43-57°C, with maxima of 75°C, 74°C, 79°C and 75°C for all the three countries (Christensen *et al.*, 2002). *Salmonella* was present in the raw material at all 4 facilities but, with the exception of the plant in Sweden where 2 of 5 samples of sanitized compost were positive, it was not present in sanitized or finished compost. *E. coli* concentrations were reduced in all facilities and numbers above the detection limit were only observed in 4 of 47 samples analysed. Since concentrations below the detection limit were observed for most of the samples the exact reduction could not be calculated. However, in general, reductions of 4.9 to 6.6 log units were achieved.

The reduction of *Enterococcus sp* ranged from 4.1 to 5.7 log units. Reduction was less efficient in windrow units. At the facility in Sweden the temperature was significantly lower at the base of the windrow and this coincided with a less efficient reduction of *E. coli* and *Enterococcus* (Christensen *et al*., 2002)

2.3 Protozoa and Helminthes

The nematode *Ascaris lumbricoides* is amongst the most cosmopolitan and most common pathogens of humans (Hannan, 1981). They can survive for a long period in the environment (Feachem *et al.,* 1983). In their fully-developed, second-stage larval form, eggs of Ascaris are highly resistant and are frequently used as indicator organisms for water and sewage treatment processes. They may, therefore, be an ideal indicator for the effectiveness of composting to reduce parasites (Mara and Cairncross, 1999). Meekings *et al*. (1996) studied the survival of *A. galli* eggs, which they used as a model of *A. lumbricoides*, in municipal waste compost and sewage sludge compost at 30^{o}C. They demonstrated that fully-developed eggs could still be observed after 20 days of composting, although these had declined from 60 per cent to approximately 12 per cent during this period. Hays (1996) reported that Ascaris ova were destroyed in a well-managed windrow composting system and Whiley and Westerberg (1969) found that the aerated pile method was also effective in destroying *A. lumbricoides* eggs, which were killed in composted sewage after 3 days at 60 to 70°C.

Other parasites and protozoa may occur in compost, but little information on their survival are available. It may be expected that the survival time of protozoa such as *Cryptosporidium* and *Giardia spp* may be extended, since both may remain viable in the environment for more than a year (Current, 1998). *Giardia* cysts were still detected after windrow composting at 52 to 53°C although it was not certain whether they were still viable (Gibbs *et al.,* 1998). In contrast, *Entamoeba histolyica* and *Endolimax nana* had disintegrated after windrow composting of municipal solid wastes after 7 days. The parasites *Necator americanus* and *Ancyclostoma duodenale* were also killed by the same conditions.

2.4 Composting Systems

There are a large number of different composting systems available, ranging in technological sophistication and therefore in cost. There are open systems (windrow and aerated static pile) which are relatively simple to operate and low cost, and a number of contained systems which have options for moving the material, supplying forced air, and operating on a continuous or batch system. Open windrow composting is the most common of the technologies currently used in the UK. It takes between 12 and 20 weeks to complete and consists of forming the mixture of raw materials into long narrow piles or windrows, which are turned and re-mixed on a regular basis. The height of windrows ranges from approximately 1 m for dense materials such as manures to approximately 3.5 m for less dense materials such as leaves. The width of windrows varies from 1.5 to 6 m with the shape determined normally by the equipment used to form the windrows.

Specialized turning machines produce low wide windrows and machines with front-end buckets produce tall windrows. The composting process depends upon a good supply of oxygen, therefore air must be able to move through the windrow. This will depend upon the size and shape of the windrow, the porosity of the material, and its water content. Feedstock material is usually shredded to ensure the correct porosity. A windrow constructed of low density materials such as leaves can be much larger than a windrow constructed of wet dense manure.

Anaerobic areas can occur near the centre of the windrow if it is too large, too dense or too wet, and these areas will release odors when the windrow is turned. On the other hand, small windrows lose heat quickly and may not achieve high enough temperatures to kill pathogens and weed seeds. Turning releases trapped heat, water vapour and gases and also mixes the materials, breaks up large particles and restores the pore spaces eliminated by decomposition and settling. Turning also exchanges the material from the outside of the windrow with that from the interior. This helps to ensure that all material receives equal exposure to the air at the surface and to the high temperatures inside the windrow thereby providing a uniform treatment process.

2.5 Occurrence of Pathogens in Human and Animal Waste and Compost

In the UK the diseases of humans which are most important are probably foodborne because there is a potential for compost to increase the risk of infection. These are *E. coli* (and particularly enteropathogenic [EPEC], enterohaemorrhagic [EHEC] and enterotoxigenic [ETEC] strains), *Campylobacter jejuni* and *C. coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Shigella dysenteriae*, (and others, including *S.*

sonnei), *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus, Clostridium botulinum, hepatitis viruses, Norwalk virus*, *Arboviruses* and Caliciviruses (Cook, 1991). Of these, the most important are *Salmonella* and *Campylobacter spp* since infections with these agents are predominant, and enterohaemorrhagic *E. coli*, particularly *E. coli O157:H7* because infections, although far less common than *Salmonella* and *Campylobacter*, are life-threatening in susceptible populations. Salmonella is endemic in poultry, cattle, pigs and sheep and pets in the UK. The organism has declined in humans and poultry but its prevalence in cattle and pigs is probably increasing. There is a great potential for the contamination of green compost if it contains faeces from farm animals, humans or pets (Andrews and Jacobson, 2001).

Infections by *Campylobacter* are the most frequent cause of enteritis in humans (Koenrad *et al.*, 1997; Tauxe, 1992). Undercooked poultry products, raw milk, untreated surface water and pets are probably the most important vectors but the organism is almost universally present in farm animals and particularly poultry. Fortunately, and unlike Salmonella and *E. coli*, this organism does not grow outside a mammalian or avian host and this may reduce the risk of disease transmission via compost, although it may play a role in maintenance of the organism in the environment.

Enterohaemorrhagic *E. coli O157:H7* (EHEC) occurs predominantly in ruminants (Stevens *et al.*, 2002) but also in wild animals, birds and domestic pets (Mainil, 1999). Wild and domestic animals may act as vectors of transmission of EHEC to farm animal hosts and, on rare occasions, humans. Indeed, wild rabbits were implicated in an outbreak of EHEC infection in visitors to a wildlife centre in England (Pritchard *et al*., 2001), probably following contamination of picnic areas with rabbit faeces.

Healthy cattle and sheep sporadically carry *E. coli O157:H7* in their gastrointestinal tract and shed the bacteria in their faeces (Chapman *et al.,* 1993; Hancock *et al.,* 1994; Kudva *et al*., 1997; Kudva *et al.,* 1996). In cattle, *E. coli O157:H7* occurs with an overall prevalence of 0.3 to 6.1per cent and the average time those faeces from an individual animal remains positive are 30 days (Sanderson *et al.,* 1995; Wells *et al.,* 1991; Kudva *et al.,* 1996). In sheep the prevalence is 0.9 to 31per cent and the organism is shed in faeces for more than a year (Kudva *et al.,* 1996; Kudva *et al.,* 1997; Zhao *et al.,* 1995). Contamination of non-ruminant food sources of infection is most often from ruminant manure (Samadpour *et al.,* 1994; Tarr, 1995; Waters *et al.,* 1994) and vegetables associated with outbreaks were found to have been grown in soil layered with manure (USDA, 1997; Cieslak *et al.,* 1993).

Boulter *et al.* (2002) carried out an extensive study of bacteria isolated from green compost. The majority of organisms were Gram-negative pathogens, or potential pathogens including Salmonella, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Bordetella bronchiseptica*. The latter is a specific pathogen of farm animals and only the organisms previously listed would indicate a possible risk to humans or animals exposed to the end product.

Pathogens of farm animals should also be considered. These include human pathogens such as *Salmonella sp* and *E. coli* but should also include diseases of cattle such as bovine tuberculosis, Johne's disease (*M. paratuberculosis*), Mucosal Disease, (Bovine Viral Diarrhoea Virus) and disease of pigs such as Swine Dysentery (*Serpulina hyodysenteriae*). Of particular importances are OIE List A diseases which do not normally occur in the UK including Foot and Mouth Disease, Classical Swine Fever and Newcastle

Disease. These and Aujesky's Disease (pseudorabies) can be transmitted via food wastes (Strauch, 1996) and are an obvious hazard.

2.6 Survival of Pathogens in Human and Animal Waste and Compost

Boulter *et al.* (2002) defined composting as "intense microbial activity leading to decomposition of most biodegradable materials (Weltzein, 1991; Adani *et al.,* 1997). This biological process involves the complete or partial degradation of a variety of chemical compounds by a consortium of microorganisms, the composition of which changes as composting progresses (Whitney and Lynch, 1996). During composting, the microbiological community follows a predictable successional pattern." Microbial activity is a prerequisite for a satisfactory composting process (Beffa *et al.,* 1996). Mesophilic, thermotolerant and thermophilic bacteria, actinomycetes and fungi are involved in the composting process. Similarly, the physical and chemical conditions involved during composting are variable and these may alter the results of the process unpredictably (Richard and Zimmerman, 1995). It is, therefore, not surprising that reports of the survival of microbial pathogens in composted wastes are variable.

The absolute time to extinction of an organism is less important than the rate at which the concentration declines. Although the former is a function of the latter the reporting of the survival time without specifying the rate of decay and decay conditions and how they may affect the rate of decline has resulted in incorrect conclusions. Obviously, the greater the number of pathogens present the longer will be their time to extinction.

Jones (1982) reviewed information on the survival of salmonellas in cattle slurry. The reduction of pathogens in sewage sludge by composting has been extensively reviewed by Dumontet *et al.* (1999). Sludge may contain a large variety of bacterial and viral pathogens including *Salmonella*, *Shigella*, *Yersinia spp*, and enteroviruses as well as eggs of parasites such as *Ascaris lumbricoides*, *Cryptosporidium* and *Giardia spp*. Competition and depletion of nutrients combined with increased temperature results in the inactivation and destruction of pathogens (Stentiford, 1986, Berney *et al.,* 2003). A composting procedure with a residence time of 3 days at a temperature of greater than 55°C resulted in a sanitized compost.

2.7 Other Bacteria

There is little information on the survival of other bacteria during composting. Gaby (1975) reported a shorter survival time for *Leptospira philadelphia* than enteric organisms. The former survived for only 2 days in windrow-composted municipal waste solids, compared to 7 to 21 days for *Salmonella* and *Shigella spp*. *Mycobacterium tuberculosis* was killed in a similar system by 14 days at 65°C (Morgan and McDonald; 1969).

2.8 Viruses

Although viruses of human and animal origin have been found in wastes (Turner *et al.,* 1999) including refuse (Golueke, 1977) their survival during waste treatment has not been well-studied. In trials in which cattle manure was inoculated with a bovine enterovirus and a bovine parvovirus (Monteith *et al.*, 1986) both were inactivated within 30 minutes of thermophilic anaerobic digestion at 55°C and neither virus survived aerobic composting for 28 days at 60°C. Similarly, Turner *et al.* (1999) reported that Swine Vesicular Disease Virus in pig slurry in an experimental pilot plant was inactivated after 5 minutes at 50 to 55°C at alkaline pH (7.5 to 8.0) and at 55 to 60°C at pH 6.4. African Swine Fever Virus was inactivated at 50°C at pH 8.

Information is available on the destruction of poliovirus by composting. Wiley and Westerberg (1969) reported that type 1 poliovirus was killed by composting of human sewage at 60 to 70°C for 3 days, whilst Gaby (1975) found that type 2 virus could not be detected after composting of municipal solids wastes for 3 to 7 days. Inactivation is probably related to temperature. Ward and Brandon (1978) demonstrated that Poliovirus was progressively inactivated at temperatures between 35 and 47°C in composted biosolids. At 47°C inactivation occurred after 5 minutes.

Because of their increased resistance it has been suggested that bacteriophage may be used as indicators of composting efficiency. Compared to other viruses and most bacteria their survival time during composting is extended. Burge *et al.* (1978) reported that the bacteriophage survived for 20 days in an aerated static pile and up to 70 days in windrows. This compared to the survival of *Salmonella sp* for 10 and 15 days respectively in the same systems.

2.9 Agents of Transmissible Spongiform Encephalopathies

Some mention should be made of the agents of transmissible spongiform encephalopathies (TSEs: Scrapie, Bovine Spongiform Encephalopathy, Creutzfeldt-Jakob Disease, v-Creutzfeldt-Jakob Disease, Chronic Wasting Disease, etc.) although these agents are not likely to be transmitted or maintained in the environment by compost (Epstein, 1997). TSEs are highly resistant to heat and chemical inactivation and their survival in the environment are probably prolonged. They may persist in soil for several years. They are not thought to be excreted in the faeces or urine of infected individuals although they could gain access to waste if infected animal tissues are not disposed of correctly. In the UK specified risk materials from cattle and sheep (SRM) are usually disposed of by incineration. The epidemic of BSE in the UK which developed in the 1980s is now declining. Scrapie is still endemic in sheep and it is not clear whether BSE has established itself in the sheep population (Baylis *et al*., 2002). TSE agents are unusual in that the infective particle may be a protein and unlike other "organisms" may not contain nucleic acid (McKinley *et al.,* 1983; Prusiner *et al.,* 1982). Their normal route of infection is by ingestion, direct injection, scarification or maternal transfer (Pattison *et al.,* 1972; Dickinson, 1976; Taylor *et al.,* 1996) but concentrations in compost are unlikely to cause a problem. They do not increase in concentration outside their host. Because of the difficulty in recovering TSE agents their presence in the environment has rarely been studied and there is no information on their survival during composting.

2.10 Standards for Pathogens in Compost

It is not the purpose of this review to discuss standards for composting of wastes or methods of assessing the efficiency of the composting processes. It has been proposed that the attainment and maintenance of a temperature higher than 55^{o}C over a 3-day period should have been sufficient to have eliminated all pathogens (Anon, 1981) and Strauch (1996) documented the requirements proposed in a number of countries. A list of organisms which could be used as indicators of the efficiency of processing has been proposed (Anon, 2000). Sanitization standards for compost have been developed in the

USA (Composting Association of the United States, 1993; Leege and Thompson, 1997; Composting Association of the United States, 2000). The latter have been reviewed recently and specify minimum compost temperatures of 55-65°C for periods of 3 to 14 days depending on the composting process (turned windrow, in-vessel, static aerated piles). A risk assessment of composting treatment to dispose of catering waste containing meat recommended a minimum composting temperature of 60°C for 2 days (Gale, 2002). This was based on the eradication data for a large number of animal pathogens. However, there may be considerable differences in composting temperatures between composting systems of the same 'category' depending on dimensions, airflow and ambient temperature (De Bertoildi *et al.*, 1996). The US EPA in "Processing to Further Reduce Pathogens" (Composting Association of the United States, 1993) established criteria for composts made with biosolids. According to the Federal Biosolids Technical Regulations, a windrow must reach a minimum temperature of 55°C for 15 days, with a minimum of 5 turnings. For an in-vessel or static pile system a minimum temperature of 55°C for 3 days is required.

2.11 Environmental Impacts

Composting is a process based on the management of microorganisms naturally present in waste materials. In addition to the positive beneficial effects of this process on the environment, there are a number of potential negative impacts. The waste material may contain within its microbial population organisms which are potentially pathogenic to humans, animals and plants.

This is currently a major concern (Gale, 2002) and should be considered further during the composting process, the generation of bye products which have potentially negative impacts on the environment, in particular gaseous bye products. Ammonia may be volatilized and released from N-rich materials by microbial activity which accumulated during the composting. Depending upon the redox conditions, microbial decomposition of organic materials will lead to the production of CO_2, N_2O, or CH_4 (Hellmann *et al.*, 1997). All of these three gases contribute to the greenhouse effect, but their net contributions to global climate change differ.

2.11.1 Ammonia

The majority of manures generated by the UK livestock industry (approximately 90 million tonnes per year) come from dairy and beef farming (73 million tonnes per year). These manures represent a significant nutrient resource of around 280,000 tonnes of Nitrogen, 50,000 tonnes of Phosphorus, and 250,000 tonnes of potassium (Smith *et al.*, 2001b). It also represents an important source of organic matter.

Manures can act as a source of pollution to water and air, with an estimated 117,000 tonnes of ammonia emissions from dairy and beef systems (DEFRA, 2002a). Emissions from organic waste heaps increase to a maximum in the first few days of storage as the bacteria cause the temperature to rise. Most of the emission from such heaps takes place within the first 30 days of storage, unless the heap is turned, or forked, to encourage composting and more ammonia is emitted. Heavy rainfall reduces emissions by slowing the composting process, but it may increase drainage from the heap and the movement of water-borne pollutants down through the soil.

There may be a further peak of ammonia emission when the heap is broken up before being spread on the land. Dairy farms are estimated to produce manures in the form of approximately 65% slurry and 35% farmyard manure, and beef units 20% slurry and 80% farmyard manure (Smith *et al.*, 2001b). Straw-based waste management systems are assumed to have less environmental impact than slurry, but preliminary results from controlled experiments suggest that total emissions of ammonia are similar for the two systems (Chambers *et al.*, 1999). Farmyard manure contains a smaller proportion of soluble nitrogen than slurry; this means that the nutrients in farm yard manure are available at a slower rate for plant uptake, and also are less susceptible to leaching (Smith *et al.*, 2002).

2.11.2 Greenhouse Gases

Carbondioxide (CO_2) is one of the products of aerobic respiration, but in contrast to CO_2 produced by fossil fuel combustion, CO_2 derived from degradation of plant materials does not contribute to global warming because it had been previously removed from the atmosphere by photosynthesis. Nitrogen dioxide (N_2O) is a product mainly of the microbial reduction of nitrate (NO_3) under anaerobic conditions (denitrification) but also to some extent via nitrification. Methane (CH4) is a product of the microbial reduction of CO_2 under anaerobic conditions. The release of N_2O and CH_4 to the atmosphere contributes to the enhancement of the greenhouse effect (Hellmann *et al.*, 1997). The flux of CH_4 to the atmosphere can be avoided if the material is subject to anaerobic digestion in which the CH_4 is collected and used as a source of energy.

Composting is an aerobic process which depends essentially on the supply of O_2 to the microorganisms within the pile, but anaerobic conditions can develop in poorly managed systems. It can be concluded therefore that well-managed aerobic composting

systems should not make a significant contribution to greenhouse gas emissions, whereas poorly managed systems which become anaerobic can contribute to N_2O and CH_4 fluxes.
Smith *et al.* (2001a) analysed the carbon balance implications of the range of waste management options for municipal waste in the European Union (EU) from 2000-2002. It was concluded that source segregation of municipal solid waste followed by recycling (for paper, metals, textiles and plastics) and composting/anaerobic digestion (for putrescible wastes) gives the lowest net flux of greenhouse gases, compared to other options for the treatment of municipal solid waste.

In comparison with land filling untreated waste, composting/anaerobic digestion of putrescible wastes and recycling of paper produce the overall greatest reduction in net flux of greenhouse of gases.

Vegetation and soils currently absorb around 40% of global CO_2 emissions from human activities. This has raised the possibility that carbon could be sequestrated through the creation of carbon sinks. The use of composted biowaste in agriculture and horticulture can also improve the organic matter content of soil and as a result soil carbon sequestration.

2.11.3 Leachate

Several studies have indicated that leachate produced from both green and non-green feedstock composting operations may contain high concentrations of pathogens, organic compounds, nutrients, and/or metals, which can negatively impact water quality (ODEQ, 2001). Management practices should be implemented to minimize the amount of wastewater produced, and recommended protection mechanisms should be constructed to protect water resources.

2.12 Effect of Composting on Chemical Parameters of the Finished Product.

Composting involves converting waste material into a product that has been stabilized and that confers beneficial effects when added to soil. The process therefore involves a range of chemical transformations mainly carried out by microorganisms. Stabilization is achieved by the conversion of readily biodegradable organic compounds into less biodegradable products, in particular humus. This may also involve the stabilization of organic forms of nitrogen and phosphorus, for example by incorporation into microbial biomass as a result these elements are less likely to be lost to the environment in leachate and gaseous emissions. Phosphorus is likely to be present largely in an organic form.

Potassium is unlikely to be changed significantly during composting. Manures contain significant amounts of potassium in the form of soluble salts which are vulnerable to leaching losses. The accumulation of salts during the composting process is due to the reduction in mass and moisture content of the material and, coupled with the formation of nitrate during the maturation phase. The resultant high salt content may cause a problem if the compost is applied to young seedlings. This is best indicated by the electrical conductivity of the material.

2.12.1 Nitrogen

Transformations of Nitrogen (N) during the composting process are important in determining the N content of the compost product and losses of N. The fate of N in feedstock materials will depend upon the biodegradability of the materials and the ratio of C/N within the biodegradable fraction (this will also affect the losses of Carbon to the atmosphere as CO_2).

Materials with a low C/N ratio such as poultry manure result in an excess of nitrogen for microbial growth and this is released as ammonium which can be volatilized into the atmosphere as ammonia gas (Finstein and Morris, 1975). Materials with high C/N ratios such as straw will not contain adequate amounts of N to support microbial growth, therefore the decomposition process will slow down. The composting process can be optimized by combining materials with complementary nitrogen contents. In extreme cases, it may be necessary to add nitrogen fertilizer to reactivate the process. During the curing stage, if aerobic conditions are maintained, the compost is re-colonized by nitrifying bacteria, and ammonium is oxidized to nitrite and nitrate (Catton, 1983).

2.12.2 pH

pH varies within a compost pile, but there is a general trend for the pH to decrease (become more acidic) during the early stages of decomposition and then to increase later (Finstein and Morris, 1975). The initial decrease in pH is due to the formation of organic acids that are formed during rapid degradation which can occur prior to composting (Hellmann *et al.*, 1997). This subsequent increase in pH is due to volatilization of organic acids, and to accumulation of ammonia.

The optimum pH for decomposition is 6.5-8.5 but in order to avoid excessive ammonia losses in aerated compost systems the pH should be <7.4 (Hoitink and Kuter, 1986). pH<5 is unfavourable for bacteria, and will prevent a number of important processes including colonization of compost by bacterial biocontrol agents (Hoitink *et al.*, 1997). If a compost pile includes a large amount of bark the low pH of this material (pH 4.5-5.2) should be balanced by the addition of ammonium-containing materials such as poultry manure to increase pH (Hoitink and Kuter, 1986). The final compost product normally has a pH of between 7.0 and 8.5 with a liming value that can help to maintain soil pH on light land.

2.13 Potentially Toxic Elements (PTEs)

The feedstock materials used for composting may contain a range of potentially toxic elements (PTEs) and limit values have been established in many countries, in many cases based on the range of values used for sewage sludge. Upper limits for conventional compost are given in the PAS 100 (BSI, 2002) and limits for organic compost derived from household waste are given in UKROFS regulations (UKROFS, 2001)

This contains a list of admissible fertilizers and soil improvers which includes pure plant and vegetable materials (plant compost, park and garden waste compost), Composted household waste was also permissible. This amendment is linked to limit values for heavy metals and the requirement that the raw material must be obtained from a closed and controlled collection and processing system. The need of farms for the compost has to be recognized by the inspection body and heavy metal limits should be lower than the PAS 100 limits values.

2.14 Organic Contaminants

The waste material used for composting may contain a large number of organic contaminants pesticides, pharmaceuticals, and industrial contaminants). The fate of these compounds during the composting process will largely determine their presence in the finished product. Only the most stable compounds are likely to persist in the compost due to the length of time taken for the composting process, the intense microbial activity, and the high temperature. For example, the pesticide diazinon which has persistence in soil of

12 weeks was not found in compost, whereas pentachlorophenol which may persist in soil for 5 years is found in compost (Deportes *et al.*, 1998).

Data on the concentration of major types of organic contaminants in composts have been reviewed by Deportes *et al.* (1998) and Buyuksonmez *et al.* (1999). Contaminants were selected based on their likelihood to cause concern due to their resistance to biodegradation, toxicity to organisms, and tendency to accumulate in the food chain (O'Connor *et al.*, 1991). Polyaromatic hydrocarbons (PAH) are stable compounds with a long half-life in soil. The total PAH content of compost ranges from 1-250 mg kg-1 with individual compounds such as naphthalene present at concentrations up to 41 mg kg-1. Following application of compost to soil none of the seven PAHs that are known carcinogens were found at a concentration exceeding the critical values (O'Connor *et al.*, 1991). Laboratory studies have shown that the co-composting of pig manure and PAH-contaminated soil caused a 90% reduction in the concentration of phenanthren and anthracene, with most of this occurring during the thermophilic phase (Wong *et al.*, 2002b).

Chlorinated hydrocarbons range from 0.02-1.1 mg kg-1 with volatile solvents present at concentrations as high as 0.1 mg kg-1. Polychlorinated biphenyls (PCBs) are highly persistent compounds and have been detected in composts at concentrations ranging from 0.0007-5 mg kg-1 (Deportes *et al.*, 1998). Organophosphate and carbamate insecticides and most herbicides were only rarely found in a study of feedstock materials and compost samples in the US (Buyuksonmez *et al.*, 1999). Organochlorine compounds are most resistant to degradation during composting. Overall it appears that composting can be considered to be essentially similar to a biologically active soil environment in which degradation is accelerated (Buyuksonmez *et al.*, 1999). However, the bioavailability of compounds may be different due to the high content of organic matter in compost, which may absorb compounds, making them less bioavailable. Little is known about the bioavailability of pesticides in compost.

The fate of a particular pesticide during composting may involve partial degradation to intermediate metabolites, volatilization, adsorption and humification, depending upon the compound, composting conditions and the microbial community. The supply of carbon and energy in composting material stimulate the breakdown of recalcitrant xenobiotics either directly or via cometabolism. Animal wastes from intensive farming and treated sewage often contain traces of therapeutic agents (compounds that are used to cure or prevent diseases). These compounds are likely to behave in the same way as pesticides in the environment, and may maintain residual activity in manure and biosolids, but have been largely overlooked (Jjemba, 2002). The quantities of therapeutic agents used in some countries are similar in magnitude to the quantities of pesticides (Hirsch *et al.*, 1999). Partially metabolized residues are excreted in faeces and urine and together with residues in leftover livestock feeds enter the waste stream. Manure and biosolids may contain residues that remain stable and survive treatment processes. The growth and development of crop plants such as *Medicago sativa* and *Zea mays* and noncrop plants such as *Plantago major* were adversely affected by sulphadimethoxine in laboratory studies (Migliore *et al.*, 1995; Migliore *et al.* 1997) and nodulation and growth of *Phaseolus vulgaris* in soil was reduced by chlorotetracycline (Batchelder, 1982).

There is a need to obtain field data on types and concentrations of pharmaceutical compounds in manure and biosolids, and in soil in order to understand potential impacts on crops in the field (Jjemba, 2002). The European Union has proposed that the concentration of pharmaceutical compounds should not exceed 10 μg kg-1 before further evaluation of a specific compound is considered (Spaepen *et al.*, 1997), but further work is needed to justify this, including studies on the impacts of these compounds on soil microbiological processes. Some countries have established limit values for organic pollutants e.g. the upper limits for PAHs are 3 mg kg-1 dry weight in Denmark an d 10 mg kg-1 dry weight in Luxembourg (Hogg *et al.*, 2002) .

In Germany and the Netherlands no limit values for organic compounds are provided because of the low level that has been detected in composts derived from the source-separated materials, only very low levels of pesticides have been detected in bio-waste and green waste in Germany and Luxembourg. In some countries regulations exist concerning the use of pesticides in gardens, partly related to concerns for the fate of these compounds once composted. Denmark, with its high rate of composting of garden waste, has recently implemented a ban on the use of garden pesticides.

In the United State there is concern that certain herbicides (e.g., chloropyralid and picloram) are very persistent to degradation, and may decompose slower in compost than in natural soils (Hogg *et al.*, 2002). Organic compounds are also derived directly from the compost itself. For example, fatty acids and methylated esters may be produced by microorganisms during the composting process. The quantities are small however, and are not considered to be dangerous (Gonzales-Vila *et al.*, 1982). Of greater concern are volatile organic compounds which cause odours, these are mainly due to sulphur compounds such as methyl mercaptan, which is produced from the amino acid methionine under anaerobic conditions (Catton, 1983).

2.15 Heavy Metals

Heavy metals are groups of metal and metalloids with atomic density greater than $4gcm^{-3}$ and are environmental pollutants (Onyari *et al.,* 1991) and could be increasingly introduced as products and finish products into dump sites and soil and have been principally associated with geochemical cycle and biological processes which could be greatly influenced by man mediated activities such as industrial activities, agricultural practices and waste disposal practice (Ubah *et al*., 2008).

Long-term field studies showed very little increase in the copper content of crops even with substantial additions of municipal solid waste (MSW) compost. The organic material in the compost binds the copper and reduces its availability to plants. Nickel concentrations are generally low in MSW composts. Thus nickel, although toxic to plants, is unlikely to decrease plant growth when compost is applied. Although applications of sewage sludge have caused toxicity to plants due to zinc, MSW composts contain much lower concentrations of zinc than does sludge. Zinc in MSW compost is unlikely to injure crops and may, in fact, be beneficial in regions deficient in zinc. However, the wide distribution of heavy metals in soil, water and atmosphere, make the raw materials for compost possible sources of heavy metal pollution (Mbarki *et al.,* 2008, Cattani *et al.*, 2008, Li *et al.,* 2007).

The content of heavy metals in compost ranges from one part per million (ppm) to one part per thousand (ppth) (Smith, 2009, Cai *et al*., 2007). Addition of heavy metals in feedstuff causes poultry manure to have high content of Cu or Zn. Excessive application of chemical fertilizers leads to the accumulation of heavy metals such as Cd, Pb or Cr in soils. In the Pearl River Delta of southern China, Pb concentrations in agricultural soils are 20%

higher than those in natural soils (Wong *et al.*, 2002). Heavy metals like Cu, Zn, Pb, and Cd varied from 7.2 to 1270 mg/kg in the municipal sludge in some cities in the middle-south region of China (Chen, 2008). To prevent the pollution of soil or groundwater by heavy metals from compost, many countries such as Belgium, Holland and Germany have established standards for heavy metals in compost. For example, in Holland, the maximum allowed concentrations of heavy metals of Zn, Cu, Pb and Cd in compost are 75, 25, 65 and 0.7 mg/kg, respectively, while in Germany, they are 400, 100, 150 and 1.5 mg/kg, respectively (Chen *et al.*, 2010).

Many studies have shown that many dump sites contain different types and concentrations of heavy metals, past investigation on the heavy metals impacts of Municipal refuse dumpsites in Nigeria were concerned with total heavy metal determination only (Bamgbose *et al*., 2000; Okoronkwo *et al*., 2006). The extent of soil pollution by heavy metals and base metal ions some of which were soil micronutrients is very alarming. It has been observed that the larger the urban area, the lower the quality of the environment (Eddy, 2004). The introduction of industrial and municipal solid waste into our environment has contributed greatly to the increase in levels of heavy metals in soil and vegetations grown in dumpsites. The soil and plants on these dumpsites will constitute a serious threat to the health of people living around such areas (Adefemi and Awokunmi, 2009).

This has been one of the reasons why problems of solid waste disposal and management have reached a critical stage in major towns and cities of Nigeria. In Nigeria, it is generally believed that individuals, government and environmental agencies pay little or no attention to the environmental impact of waste disposal and management, even when it is a statutory responsibility of the parties concerned. Agencies like the Federal Environmental Protection Agency (FEPA), Ministry of Environment, State Environmental Protection Agency (SEPA) and even local authorities are responsible for planning a defined line of action for disposal and management of waste generated on daily basis in our society, unfortunately, they have failed in this regard.

2.16 Effect of Composting on the Biological Parameters of the Finished Product

Compost is a biological product (unlike peat-based products) which contains a wide range of microorganisms that are beneficial to plants and the environment. The feedstock materials for composting are generally wastes from a wide range of agricultural, municipal and industrial sources, and in many cases these materials will contain a number of microorganisms which are pathogenic to humans, animals and plants.

Feedstock materials may also contain weed seeds e.g. from animal feeds or bedding material. The thermophilic conditions during composting should eliminate weed seeds. The PAS 100 (BSI, 2002) indicates that there should be no more than 5 weed propagules per liter of compost product.

Composting is an intense thermophilic microbial process which when managed correctly will provide a microbiologically-rich product which is safe from pathogens. There has been concern over the fate of human pathogens in sewage sludge (ADAS, 2001), and more recently attention has been focused on the risk associated with composting catering waste containing meat. A risk assessment has been carried out for DEFRA (Gale, 2002) and a draft statutory instrument published for consultation (DEFRA, 2002b) which specifies the processing systems and parameters that can be used for the composting of catering waste.

CHAPTER THREE

3.0 MATERIALS AND METHODS.

3.1 The Study Area

Zaria is located on a plateau at a height of about 2200 feet above sea level in the centre of Northern Nigeria and about 400 miles away from the sea. Zaria (11^o 3^1 N, 70 42^1 E) possesses a tropical continental climate. The tropical climate is more pronounced during the dry season, especially in the months of December and January. The mean daily maximum temperature (^oC) shows a peak in April and a minor one in October. Zaria lies within a region which has a tropical savanna climate with distinct wet and dry seasons. The tropical annual rainfall in Zaria is not high (Mean is about 44.4 inches). The vegetation of the area assumes various shades of green in wet season and turns brown, pale or yellow in the dry season.

The total population of Zaria according to the 1991 population census is put at 369, 800. Sabon-Gari being the largest settlement has a population of over 36,000; and Zaria city has over 21,000 inhabitants. Most inhabitants of these settlements dispose their domestic waste in open refuse dumps scattered across Zaria metropolis (Yahaya, 2007). The study area has two major seasons, the dry and the wet season. The dry season is basically a tropical continental climate between the months of October and April with the maximum temperature(^oC) in April while the rainy or wet season start in the month of May to September with mean maximum rain fall of 44.4 inches (Mortimore, 1970).

MAP OF STUDY SHOWING SAMPLING AREAS.

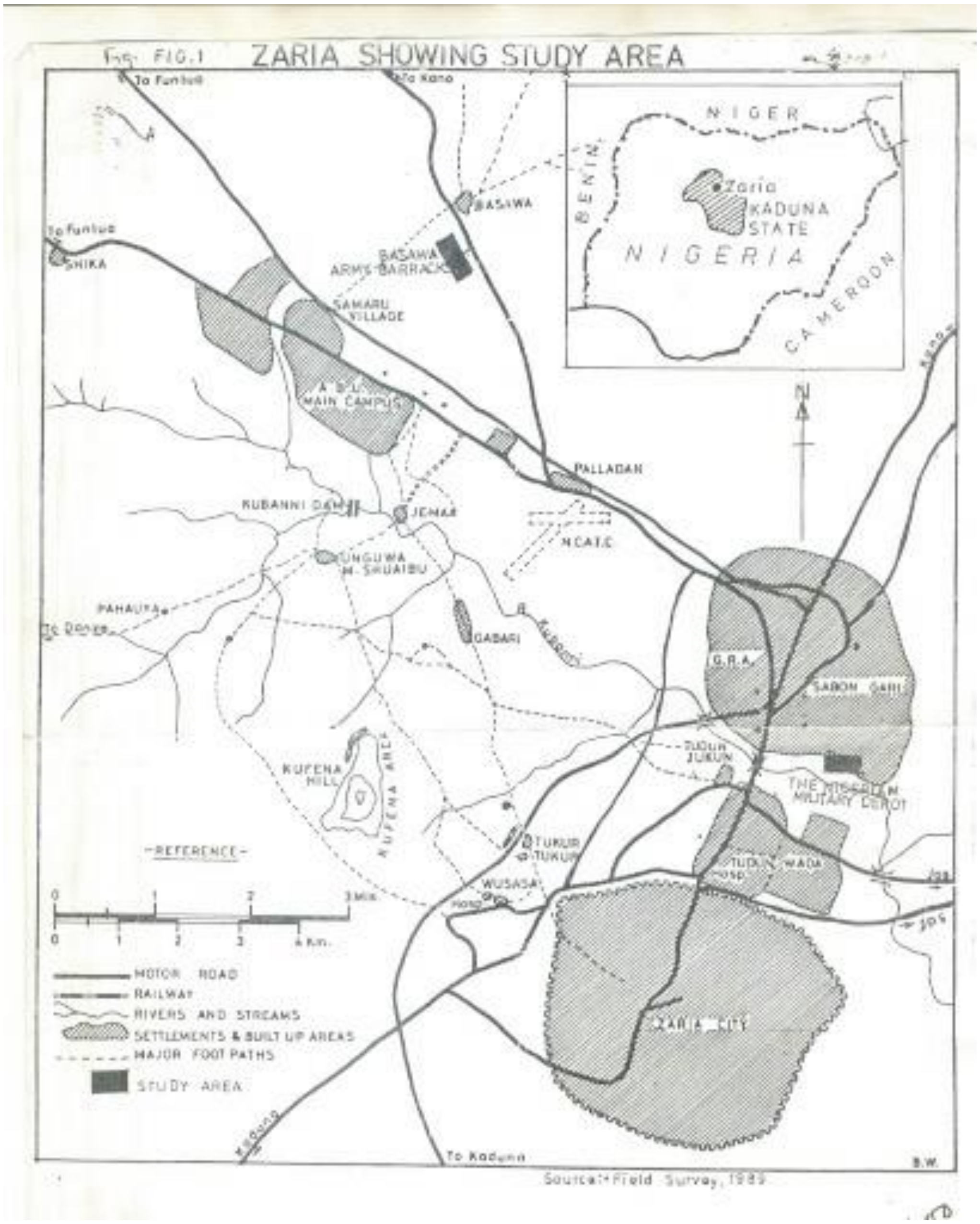

Figure1.

3.2 Sampling Sites

Sampling sites were carefully selected based on the proximity to populace because they are the major receiver of waste, from available cattle ranches and poultry farms and municipal solid waste from and around Zaria metropolis (Plates 6, 7, 8, 9 and 10) after a rough census of the various types were taken from the various parts of Zaria(appendix i).

3.3 Collection of Samples

The types of wastes collected and the quantities collected were as follows.

i. Municipal solid waste (50kg)

ii. Cattle waste (50kg)

iii. Poultry waste (50kg)

The waste samples were collected for composting using a shovel and a clean container that can carry up to 50kg of each waste to the composting site (was sufficient for proper composting). Each waste type was composted for 12 weeks each. (Samples were collected on weekly for the 12 weeks) A total of 432 samples were collected within the said period.

3.4 Composting Techniques.

The static aerated piles and windrow methods were used to compost the various waste types. It took between 12 and 20 weeks to complete and consisted of forming the mixture of raw materials into long narrow piles or windrows, which were turned and re-mixed on a regular basis. The height of windrows ranged from approximately 1 m for dense materials such as manures to approximately 3.5 m for less dense materials such as leaves.

3.5 Laboratory Samples

The composting samples were collected in sterile stainless spoon to a depth of 3cm into a sterile conical flask. These were transported in a cold pack to the laboratory for analysis that was carried out within 6 hour of collection. Samples were analysed in the Department of Microbiology, Ahmadu Bello University, Zaria.

3.6 Determination of Physicochemical Parameters of the Waste Samples

The following physicochemical parameters were determined for various solid and animal wastes.

3.6.1 Temperature

Temperature measurements were determined with a mercury-filled Celsius thermometer. The thermometer was immersed in the various wastes long enough to permit accurate and stable reading and the results were recorded. The thermometer was carried along to the sampling sites and readings taken directly from the samples. ((Zeal, England)).

3.6.2 pH

An electronic titrator or pH meter was used and before taking readings, it was standardized according to the manufacturers' instruction. The standardized pH meter was carried along to the sampling sites and the electrode immersed in the various samples enough to permit accurate reading of pH. (HI991405, Hanna, UK).

3.6.3 Conductivity

The self conductivity instrument was used (LM8, Norylab). It is an instrument capable of measuring conductivity with an error not exceeding 1% ohm/cm. To measure conductivity, the measuring cell was cleaned thoroughly and rinsed with distilled water or sample to be measured. The cell was filled with samples and plugged to the connecting cables case into the measuring cell socket. Temperature dial was set to the temperature of the samples and the selector switch was set to the anticipated range of measurement. The activator button was held down slowly and the measuring dial rotated until the balanced indicator moved to the centre scale. The dial setting was read, and was multiplied by the range factor. The result was expressed as samples specific conductivity in micro ohms/cm.

3.6.4 Determination of Heavy Metals in Waste

All compost samples collected were air-dried and ground to pass through a 1 mm stainless sieve, they were then stored in plastic bottles prior to analysis.

3.6.5 Compost Sample Digestion

The various waste samples (0.5g) were weighed each into 100cm^3 beaker, a mixture of 5 cm^{3}concentrated HNo_3 and 2 cm^3 of $HClO_4$ were added and these were digested on low heat using hot plate until the content was about 2 cm$^{3.}$ The digest were allowed to cool and then filtered using Whatman No. 42 filter paper and less than 0.45µm Millipore filter paper into a 500 cm^3 standard flask. The sieved samples (5g) each were accurately weighed into a 100 cm^3 beaker and 10 cm^3 concentrated HNO_3 was added to each. The mixture was with a watch glass and refluxed for 45minutes. The watch glass was removed and the contents in the beaker were allowed to evaporate to dryness. Aqua-regia

($10 cm^3$) was added to each sample and evaporated to dryness after $10 cm^3$ 1M nitric acid was added and the suspension was filtered using Whatman no. 42 filter paper. The filtrates were then diluted to $50 cm^3$ with distilled water in volumetric flask (Sposito *et al.*, 1983).

The concentration of Lead (Pb), Nickel (Ni), Zinc (Zn), Cadmium (Cd) and Copper (Cu) were determined using the Atomic Absorption Spectrophotometer Model (Shimadzu Double Beam Digital AAS-650) at Department of Chemistry, Ahmadu Bello University, Zaria.

3.7 Microbiological Analyses of the Waste Samples

3.7.1 Preparation of Media

All media used were prepared from commercially available powder, and manufacturer's instructions were followed. Sterilization of these media was achieved by autoclaving at 15 lb / sq inch and 121^{o} C for 15 – 20 minutes. Pipettes and other glassware were packed in canisters and sterilized in the hot air oven at $160^{o}C$ for 90 minutes. Commercially available media were used after confirmation of their sterility by leaving prepared plates on bench for 24 hours. Aseptic conditions were maintained throughout the period of sample analysis to prevent contamination of samples and media.

3.7.2 Determination of Total Bacterial Count

Twenty five grams of composting waste sample collected was weighed into 225ml of sterile distilled water (10^{-1}). Samples were serially diluted (10^{-1} to 10^{-6}). The last two dilutions (10^{-5}, 10^{-6} were inoculated in duplicates on the plate count agar medium (PCA) using the pour plating method. Total bacterial counts were determined after incubation at $37^{0}C$ for 24 hours (Sikora, 1983).

3.7.3 Total Coliform Count

The total coliform count was determined using EMB (Eosin methylene blue agar). Twenty five grams of composting waste sample collected was weighed into 225ml of sterile distilled water (10^{-1}) and further diluted serially (10^{-1} to 10^{-6}). The last two dilutions (10^{-5} to 10^{-6}) were plated out in duplicates using the pour plating method. The plates were incubated at 37°C for 24 hours after which the total coliform counts were determined (Sikora, 1983).

3.7.4 Isolation of *Escherichia coli* from Samples

Escherichia coli was isolated from samples using tryptic soy broth (TSB) as pre-enrichment and incubated at a temperature (44.5° C). A total of 20 ml of samples were combine with equal volume of sterile double strength Tryptic soya broth in cotton plugged 150 ml Erlenmeyer flask and incubated at 44.5°C for 24 hours.

After the enrichment, samples were streaked on EMB agar, and incubated at 44.5°C for 24 hours. Typical lactose – fermenting, small, dark colonies with almost black centers, and greenish metallic sheen were subcultured on nutrient agar slants and incubated at 44.5°C for 24 hours and stored in a refrigerator as stock cultures for further identification biochemically (Lejeune *et al.*,2001).

3.7.5 Identification of *E.coli* O157:H7 Strains

Biochemically confirmed *E.coli* strains were screened with Sorbitol MacConkey (S.M.A.C., Oxoid, Basingstoke, UK) agar plates. After incubation at 37° C for 24 hours, all non-Sorbitol- fermenting colonies exhibiting morphology typical of the *E.coli* O157:H7 (colourless, gray or pale with a darker centre) were recorded as presumptive *E.coli* O157:H7 (Agbogu, 2004).

3.7.6 Agglutination test for *E.coli* 0.157:H7

The *E.coli* latex test Oxoid serocheck and seroscreen tests kits which use antibody sensitized blue particles dried on cards was used. The latex agglutinates in the presence of specific *E.coli* cell wall H-antigens was observed to form visible clumps (Agbogu, 2004).

3.7.7 Isolation of *Vibrio cholerae*

One milliliter of the stock sample in alkaline peptone water (pH 8.6) was incubated at 35-37^{o}C for 5-8 hours. This was subcultured into thiosulphate citrate bile salt (TCBS) agar, and incubated aerobically at 35-37^{o}C overnight for isolation of *Vibrio* sp (Cheesbrough, 2002)**.**

3.7.8 Agglutination tests *Vibrio cholerae*

The *Vibrio cholerae* isolated and identified were confirmed serologically by the slide agglutination technique using *Vibrio cholerae* antiserum poly ABC and further characterized as Inaba or Ogawa (Yahaya, 2007)

3.7.9 Isolation of *Salmonella spp*

Five milliliter of the stock sample was mixed with 45 ml of buffered peptone water (BPW) as pre-enrichment for *Salmonella* and incubated for 24 hours at 37^{o}C. The pre-enriched cultures (1ml) were subcultured in 9 ml selenite F broth for selective enrichment (Kent *et al.,* 1981). These were plated on Bismuth sulphite agar. All black colonies with metallic sheen were subcultured on slant for further biochemical characterization (Yahaya, 2007).

3.7.10 Agglutination test for *Salmonella typhi*

The *Salmonella* latex test, Oxoid FT10203 kit for rapid agglutination test for the presumptive identification of *Salmonella typhi* in selective and enriched cultures were used.

3.7.11 Detection of Helminths / eggs / larvae in Compost.

The concentration technique and wet mount method were carried out. The formalin ethyl acetate sedimentation concentration procedure as described by Habbari *et al.* (1999) was followed. Using the recommended speed and time (500rpm for 10 minutes), 1 g of sample was mixed with 9 ml of neutral buffered 10 percent formaldehyde solution and then with 4 ml of ethyl acetate. Samples after shaken in inverted position for 30 seconds and centrifuged at 500 rpm for 10 minutes. Sediments were examined by tapping the tube at the bottom before dropping with Pasteur pipette on a glass slide. Ova were identified according to the key proposed by WHO (1993). Counting of helminthes was done by direct microscopy using the 40× objective.

3.8 Molecular Characterization of Bacterial Isolates

3.8.1 Extraction of DNA

The DNA of the target bacterial species were extracted. The process involved the centrifugation of 1.2ml cells in micro centrifuge for 15seconds and re-suspended in 0.3ml HTE buffer (50mM, Tris-Cl, pH8.0, 20nm EDTA). A total of 0.35ml 2% Sarcosy in the buffer were mixed by inversion of tube. DNA (5γl Rnase, and incubate at 37^{o}c for 15 minutes. A total of 35µl of pronase was added and heated at 50^{o}C (until lysis is complete (30-90 minutes) (Munirul *et al.*, 2006). With the aid of maximum speed setting and vortexed lysate for two minutes to shear the DNA. In extracting the DNA, an equal volume (0.70ml) of the phenol, chloroform 1:1 was vortexed briefly (5-10 seconds) to mix, and then centrifuged at full speed in micro centrifuge for 10 minutes to separate the phases, and the top layer was carefully removed with sterile Pasteur pipettes and added to a new micro centrifuge tube (it was ensured that flocculent inter phase is taken along). The aqueous DNA was re extracted with phenol chloroform one time, before disposing off the phenol (Kay *et al.*, 1994).

Further extractions were done with one volume of chloroform isoamyl alcohol, after which the DNA was precipitated by adding sodium acetate 0.3M. (70µl) and mixed well before adding equal volume of isopropanol (0.7ml). After the mixture was inverted in the tube 5 to 6 times before freezing in an ethanol dry ice bath for ten minutes or longer before removing supernatants with a drawn out Pasteur pipettes. The DNA pellet were obtained by adding 1.0ml of 70% ethanol, vortexed briefly and centrifuged for 5 minutes and removed supernatants with a drawn out Pasteur pipettes. These were repeated twice to remove residual salts as well as remaining phenol and chloroform. The two were rapped with paraffin, poked hole with needle and dry pellets under vacuum to remove ethanol, over drying was avoided, the precipitated DNA will be dissolved in 50µl “T H E” buffer and labeled and stored at -20^{o}c for further use (Kamalendu, 1990).

3.8.4 Restriction Endonuclease Digestion of Chromosomal DNA.

The DNA isolated previously (2µg of chromosomal DNA) was used for the agarose gel as the undigested control for the next protocol, and also leaving 0.5mg for the PCR experiment. The following order below was followed in adding components of the restriction digest reaction mixture to microfuge tube. (final volume of 50µl) and 41µl of sterile distilled water.

a. 5µl of 10x Bam HI Buffer (500mM, Trs-Cl, pH 8.0, 100mM $MgCl_{2,}$ 1000mM NaCl).

b. 3µl of chromosomal DNA

c. 1µl of Bam HI restriction endonuclease (5 units) or any commercially available restriction endonuclease for *Vibrio, E.coli 0157.H7 and Salmonella* spp.

The mixtures were allowed to digest for 2 hours or overnight at 37^{o}C, this was stored in a -20^{o}C freezer until required for use.

3.8.5 Agarose gel of Chromosomal DNA, Restriction Endonuclease Digestions.

The outside of the gel former was seated with tape. The comb was positioned about 1mm above the surface of the frame by placing an index card between the bottom of the comb and the gel plate. After removing the index card, about 0.8% agarose gel was poured into the gel frame, after hardening completely of the gel (20-30 minutes) the comb was removed gently, making sure gel tray is leveled to prevent the gel from sliding. The gel was transferred to the electrophoretic apparatus and covered with IXTEA buffer (perlitre, 48.4g of trisma base, 11.42ml of glacial acetic acid, 20ml of 0.5M EDTA, pH 8.0). The DNA samples were mixed with a droplet of loading buffer on paraffin that had a hydrophobic surface. For the undigested sample (control), 2µl of DNA with 2µl of 6X loading buffer on paraffin was used. The 0.5 µg of the 2kb ladder were loaded in 1 well, after loading wells with samples the electrophoresis apparatus was connected to power supply and turned on the power supply (Herrmann and Frischauf, 1987).

3.8.6 Staining and Photography of Agarose gel of Chromosomal DNA Restriction Endonuclease Digestions.

The gel was stained for 20minutes in the ethidium bromide solution and detained in water for 20 minutes. The ultra violet radiation (u/v) trans illuminator box was made wet with water and covered with plastic wrap to prevent ethidium bromide from staining the u/v box. The gel was moved carefully into the u/v box using the protective glasses and the u/v light was turned on and the gel was examined, photographed with the Polaroid camera. The photograph were used to determine the distance migrated by each of the band in comparison with the 1kb ladder (Kohara *et al.,* 1987).

3.8.7 Polymerase Chain Reaction Detection of *E.coli 0157: H7, Salmonella typhi, Vibrio cholerae* 16 rDNA Gene Regions for Prokaryotes

A Polymerase chain reaction assay targeting 16S intergenic spacer region specific for prokaryotes using methods described by Lane, (1991); Hoshino *et al*., (1998) were used. Whereas the genes were amplified with 27F and 1492R primers which amplified all 16S regions for all prokaryotes (Lane, 1991; Kay *et al.,* 1994; Turner *et al.,* 1999b), For the PCR amplification, samples were enriched by incubation in APW (Difco) at 37^{o}c for 6 to 8 hours and pre-incubated with 0.025% yeast extract (Difco) and 0.002% nalidixic acid. The broth cultures were centrifuge at 10, 000rpm to collect cell pellets. DNA was extracted from the pelleted cells and subjected to PCR as described by(Munirul *et al.,* 2006).

Amplification with the three primer pairs was performed simultaneously in 0.2ml micro centrifuge tubes. Samples (3µl) were added to the PCR mixture (final volume 30µl) containing each deoxynucleaside triphosphate at a concentration of 0.21mM, 50mM KCl, 1.5mM $MgCl_2$, 10mM Tris-HCl (pH8.3), each member of the primer pairs at a concentration of 0.27µM, and 0.75U of *Taq* polymerase. The amplification condition used was five minutes at 94^{o}C for initial denaturation of the DNA, 35 cycles consisting of 1 minute at 94^{o}C, 1 minute at 55^{o}C, and 1 minute at 72^{o}C in a DNA Robo cycler gradient temperature cycler (Stratagene, Lajolla,CA). After amplification, 6µl of each reaction mixture were separated by electrophoresis on a 3% agarose gel, and the amplified gene products were visualized under u/v light after staining with ethidium bromide.

3.8.8 Enterobacterial Repetitive Intergenic Consensus PCR

This method was used for distinguishing intra specific variations among bacterial strains of the different species using PCR primers derived from within sequences. The isolates repetitive intergenic consensus sequences palindromes occurred in multiple copies in the genomes of enteric bacteria and Vibrio. They were distributed randomly in the bacterial species throughout the whole genome. The primers were designed so that amplification occurred between copies of the sequence. If the positions of copies varied among different strains, the amplification products provided each strain with a unique fingerprint when run on a gel.

PCR was carried out using universal primers 27F(5′- AGAGTTTGATCMTGGCTCAG-3′) and 1492R(3′-GGTTACCTTGTTACGACTT-5′), in 25µl volume using 10ng of DNA, 1U of Taq polymerase (Inqaba, South Africa), and 10pmoles of each primers (Inqaba, South Africa), 0.25mM (each) deoxynucleotide triphosphate (MBI, Fermentas) and 1.5 mM MgCl2 in standard PCR buffer.

3.9 Antibiotic Susceptibility Testing

3.9.1 Preparation of Inocula

The antibiotic susceptibility patterns of the isolates were determined by using the Kirby-Bauer NCCLS Modified disk diffusion techniques. The inoculums of each organism were prepared by standardizing overnight nutrient broth (Oxoid) cultures of the isolates using the 0.5McFarland turbidity standard (NCCLS, 2000).

Cotton swab was dipped into the suspension, rotated several times and then streaked over the entire surface of already prepared Mueller Hinton agar (Oxoid) plates and allowed to stand for 15 minutes to for excess surface moisture to be absorbed. Antibiotic multi disc (Oxoid) comprising of Peflacine (10mg-l), Ciproflox (10mg-l), Augmentin (30mg-l), Gentamycin (10mg-l), Streptomycin (30mg-l), Ceporex (10mg-l) Nalidixic acid (30mg-l), Septrin (30mg-l), Ampicillin (30mg-l) and Tetracycline (30mg-l) were placed on the inoculated plates with a sterile pair of forceps and gently pressed onto the agar surface to provide uniform contact. The plates were incubated at 37 ^{o}C for 18 hours.

3.9.2 Determination of Zone of Inhibition

The diameters of the zone of inhibition were measured to the nearest whole millimeter with a ruler on the back of the inverted Petri dishes. The zone diameters measured around each disk were interpreted on the basis of guidelines published by NCCLS and the organisms were reported as susceptible, intermediate or resistant to the antibacterial agent teste (CLIS, 2005). A type strains was used as control.

3.10 Statistical Analysis

The results obtained from this study were analyzed using the ANOVA and Duncan multiple range test and the levels of significance for the various parameters where determined.

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Parameters

The variations in the mean values of physicochemical parameters and the standard deviation of the compost were shown on Table 1. The mean temperature values for all the compost were 40^{o}C except that of compost 3 which was 39.65^{o}C. All the compost had mean pH values of pH>8 except compost 2 and 6 that had approximately pH7.8. The conductivity values were highest in compost 1, 4 and 6. The results revealed a significant difference between the composts. The variations in their physicochemical parameters for the 12 weeks are shown on Table 2, and the mean with superscripts are significantly different. Temperature values were <40^{o}C in weeks 1, 2, 10, 11 and 12, pH values were less than > pH 8 between weeks 3 to 7 and the conductivity values were > 700 (µs/cm) between weeks 10 to 12.

In this work, the mean total plate counts for cow, poultry and municipal solid waste was 6.30 cfu-g (log_{10}) and the mean coliform count was 5.05 cfu-g (log_{10}). The mean temperature total for cow, poultry and municipal solid waste was 40.43^{o}C and the mean pH was 8.024 and mean conductivity values was 870.37. There were significant differences in the temperature, pH, and conductivity among the different waste types ($P<0.05$; 0.032, 0.000, 0.000) respectively (Table 3).

Table 1. Comparison of the Mean and the Standard Deviation for the Physicochemical Parameters of the Compost.

Parameters	Compost	No. of samples	Mean	±	S. D
Temperature (°C)	COMPOST1	72	40.69[a]		2.954
	COMPOST2	72	40.01[a]		2.336
	COMPOST3	72	39.65[a]		2.877
	COMPOST4	72	40.85[a]		2.766
	COMPOST5	72	41.02[a]		2.748
	COMPOST6	72	40.33[b]		5.374
	Total	432	40.43		3.346
pH	COMPOST1	72	8.140[a]		0.7371
	COMPOST2	72	7.797[a]		0.6955
	COMPOST3	72	8.094b[b]		0.5561
	COMPOST4	72	8.225[b]		0.8003
	COMPOST5	72	8.128[b]		0.8192
	COMPOST6	72	7.761[b]		0.7469
	Total	432	8.024		0.7482
Conductivity (µs/cm)	COMPOST1	72	943.89[a]		355.027
	COMPOST2	72	788.19[a]		436.498
	COMPOST3	72	788.75[a]		409.427
	COMPOST4	72	958.47[b]		324.427
	COMPOST5	72	817.08[b]		429.967
	COMPOST6	72	925.83[b]		331.725
	Total	432	870.37		388.734

Means with different superscript are significantly different ($P<0.05$) using ANOVA and Duncan's multiple range test.
N= number of samples. S.D= Standard deviation.

Table 2. Comparison of the Mean and the Standard Deviation for the Physicochemical Parameters of the Compost by 12 Weeks.

Parameter	Compost	N	Mean	±	S.D
Temperature (ºC)	WEEK1	36	36.40[a]		1.788
	WEEK2	36	38.68[a]		0.803
	WEEK3	36	40.56[b]		1.081
	WEEK4	36	41.68[b]		1.609
	WEEK5	36	43.10[c]		1.735
	WEEK6	36	44.15[c]		1.357
	WEEK7	36	43.93[d]		1.665
	WEEK8	36	42.76[d]		1.391
	WEEK9	36	40.67[e]		1.095
	WEEK10	36	38.00[f]		5.880
	WEEK11	36	37.82[g]		0.575
	WEEK12	36	37.36[h]		0.733
	Total	432	40.43		3.346
pH	WEEK1	36	7.694[a]		1.1148
	WEEK2	36	7.936[b]		0.9187
	WEEK3	36	8.125[b]		0.8637
	WEEK4	36	8.394[b]		0.7266
	WEEK5	36	8.528[b]		0.6772
	WEEK6	36	8.322[b]		0.6672
	WEEK7	36	8.042[bc]		0.6967
	WEEK8	36	7.978[bc]		0.6366
	WEEK9	36	7.875[bc]		0.5714
	WEEK10	36	7.864[c]		0.4958
	WEEK11	36	7.783[d]		0.4286
	WEEK12	36	7.750[e]		0.4130
	Total	432	8.024		0.7482
Conductivity (µs/cm)	WEEK1	36	1,158.33[a]		226.154
	WEEK2	36	978.89[a]		309.874
	WEEK3	36	933.61[a]		324.831
	WEEK4	36	972.78[b]		329.351
	WEEK5	36	919.17[b]		355.635
	WEEK6	36	793.33[b]		394.230
	WEEK7	36	790.28[b]		405.966
	WEEK8	36	808.33[b]		416.849
	WEEK9	36	802.50[b]		424.825
	WEEK10	36	765.83[b]		418.831
	WEEK11	36	762.50[b]		419.022
	WEEK12	36	758.89[c]		419.072
	Total	432	870.37		388.734

Means with different superscript are significantly different ($P<0.05$) using ANOVA and Duncan's multiple range test. N= number of samples. S.D= Standard deviation.

Table 3. The Mean Effect for Each Parameter on the Compost

	Types	N	Mean ±	S. D
Total Plate Counts (log_{10} cfu-g)	COW	144	6.28	1.585
	POULTRY	144	6.19	1.539
	MSW	144	6.43	1.465
	Total	432	6.30	1.530
Total Coliform Counts (log_{10} cfu-g)	COW	144	5.08	2.453
	POULTRY	144	4.95	2.475
	MSW	144	5.11	2.332
	Total	432	5.05	2.416
Temperature (°C)	COW	144	40.37	4.163
	POULTRY	144	40.45	2.825
	MSW	144	40.45	2.903
	Total	432	40.43	3.346
pH	COW	144	8.320	0.5478
	POULTRY	144	7.414	0.7358
	MSW	144	8.339	0.5319
	Total	432	8.024	0.7482
Conductivity (µs/cm)	COW	144	739.86	323.469
	POULTRY	144	1,296.32	41.590
	MSW	144	574.93	247.680
	Total	432	870.37	388.734

Means within each group with their level of significantly different using ANOVA and Duncan's multiple range test are: plate count $p > 0.05$ (0.418), Coliform count ($P < 0.05$; 0.174), Temperature ($P < 0.005$; 0.032), pH ($P < 0.05$; 0.000), conductivity ($P < 0.05$; 0.000). N= number of samples. S.D= Standard deviation. MSW= municipal solid waste.

The mean value for the windrow and the open pile methods of composting revealed a steady decline in total plate and coliform counts. There was no significant difference between the methods of composting on their plate count, pH and temperature values ($P>0.05$; 0.229, 0.241; 0.096,), but showed significant differences between their total coliform count, temperatures, and conductivity ($P<0.05$; 0.002, 0.002) respectively (Table 4). There were lower temperatures for the first one week which later increased to a maximum mean temperature of 43.4^{o}C for the windrow and 45.0^{o}C for the open pile method of composting, the temperature increase lasted for five and six weeks respectively and later decreases with the last three weeks of composting. There was a significant difference between the windrow and the open pile temperatures ($P< 0.05$; 0.006) (Table 5).

The different waste types similarly showed lower temperatures at the initial and later part of the composting process. The mean temperatures for the cow and poultry waste peaked at 43.7 ^{o}C and 43.9 ^{o}C respectively while municipal solid waste was 44.3 ^{o}C. These peak mean temperatures were achieved at week six (6) for all the compost types after which temperatures dropped steadily to the end of composting. There was no significant differences between the different waste types composted ($P>0.05$, 0.923) (Table 6).

The windrow method showed lower pH values at the first three weeks which rise to pH 8.5 (mean), this decline steadily in the last four (4) weeks. The pH was slightly higher and steady at the week 4 and 5 of composting for the open pile, it decline steadily to the end with mean pH 7.8. There were no significant differences between the pH of the windrow and the

Table 4. The Mean Values for all the Parameters on the Composting Techniques Used

	LOCATION	N	Mean	± S.D
Total Plate Count (log_{10} cfu-g)	WINDROW	216	6.26	1.554
	OPEN PILE	216	6.34	1.508
Total Coliform Count (log_{10} cfu-g)	WINDROW	216	4.95	2.472
	OPEN PILE	216	5.15	2.360
Temperature (°C)	WINDROW	216	40.14	2.584
	OPEN PILE	216	40.71	3.950
PH	WINDROW	216	7.982	0.7470
	OPEN PILE	216	8.066	0.7488
Conductivity (µs/cm)	WINDROW	216	910.32	369.024
	OPEN PILE	216	830.42	404.406

Means within each group with their level that are significantly different using ANOVA and Duncan's multiple range test are: plate count P> 0.05; 0.229), pH P>0.005; 0.241), Coliform count P >0.05; 0.096), Temperature (p<0.05; 0.002), conductivity (P<0.05; 0.002). N= number of samples. S.D= Standard deviation.

Table 5. The Mean Temperature (ºC) Values for the Various Composting Techniques Used

LOCATION	Windrow			Open pile		
	Mean	±	S.D	Mean	±	S. D
Week1	36.4^{a}		1.803	36.4^{a}		1.825
Week2	38.5^{a}		0.795	38.8^{a}		0.804
Week3	40.7^{b}		0.942	40.4^{b}		1.216
Week4	41.5^{b}		1.366	41.9^{b}		1.836
Week5	42.7^{c}		1.871	43.5^{c}		1.519
Week6	43.4^{c}		1.346	44.9^{c}		0.862
Week7	42.9^{d}		1.539	45.0^{c}		1.021
Week8	41.9^{d}		1.198	43.7^{d}		0.907
Week9	40.3^{e}		0.927	41.1^{e}		1.136
Week10	38.7^{f}		0.569	37.3^{e}		8.361
Week11	37.6^{f}		0.530	38.0^{f}		0.555
Week12	37.3^{g}		0.752	37.4^{f}		0.725

Means with different superscript are significantly different ($P<0.05$) using Student t- test and Duncan's multiple range test. There was a significant difference between the windrow and the open pile temperatures ($P< 0.05$; 0.006). N= number of samples. S.D= Standard deviation.

Table 6. The Weekly Mean Temperature Values for the Various Wastes Composted.

Week	MEAN VALUE		
	Cow	Poultry	Municipal solid Waste
Week 1	36.333	36.250	36.625
Week 2	38.750	38.792	38.500
Week 3	41.042	40.375	40.250
Week 4	42.000	41.625	41.417
Week 5	43.083	43.125	43.083
Week 6	44.167	43.958	44.333
Week 7	43.708	43.917	44.167
Week 8	42.750	42.875	42.667
Week 9	40.917	40.625	40.458
Week 10	36.250	38.750	39.000
Week 11	38.000	37.792	37.667
Week12	37.417	37.375	37.292

There was no significant differences between the different waste types composted ($P>0.05$, 0.923).

open pile methods ($P>0.05$, 0.118)(Table 7). The pH of the various waste types composted showed significant differences with the municipal solid waste having the highest pH 8.98 and followed closely by cow waste pH 8.80. The pH for cow waste remain higher all through the composting period with pH values remaining greater than pH> 8 and poultry waste remains lower at pH >7 and never reach pH 8 throughout the composting period. The results revealed strong significant differences among the waste types composted ($P< 0.05$; 0.000) (Table 8).

There were higher conductivity values for the first four (4) weeks of composting which decline steadily after the fourth week to the end of the compost in the windrow method. The conductivity values were lower in the open pile method after the first week of composting and remain lower than those of the windrow except at week six (6) where conductivity values for the windrow method of composting become lower and went up the following week to the end of composting (Tables 9), There were a significant difference between the conductivity values of the windrow and the open pile methods of composting ($P<0.05$; 0.000). The conductivity values for the poultry waste remain higher than those of cow and municipal solid waste all through the composting period (>1million µs/cm) (Table 10) and showed significant differences between the various wastes composted ($P<0.05$; 0.000).

The concentrations of the six heavy metals in the final compost were shown on Table 11. The mean concentrations (ppm) range for the different types of waste composted were as follows: Pb 0.014 (PW) –0.0507 (MSW), Cu 0.201 (cow) –0.556 (poultry), Ni 0.048 (cow) –0.156 (MSW), Zn 2.054(Cow)–15.937 (MSW), Co 0.239(poultry) - 0.423(MSW), Cd 0.001(Cow) - 0.013(MSW). The municipal solid waste had significantly higher values for

Table 7. The Mean pH Values for the Composting Techniques Used

LOCATION	Windrow			Open pile		
	Mean	±	S. D	Mean	±	S. D
Week1	7.694[a]		1.131	7.694[a]		1.131
Week2	7.739[a]		0.927	8.133[b]		0.892
Week3	7.978[a]		0.827	8.272[b]		0.898
Week4	8.244[a]		0.827	8.544[b]		0.596
Week5	8.467[b]		0.698	8.589[b]		0.670
Week6	8.311[b]		0.704	8.333[b]		0.649
Week7	8.094[b]		0.661	7.989[c]		0.746
Week8	8.011[c]		0.677	7.944[c]		0.611
Week9	7.894[c]		0.579	7.856[c]		0.580
Week10	7.839[c]		0.495	7.889[c]		0.510
Week11	7.767[c]		0.438	7.800[c]		0.431
Week12	7.750[c]		0.405	7.750[c]		0.433

Means with different superscript are significantly different ($P<0.05$) using the student t-test and Duncan's multiple range test. There were no significant differences between the pH of the windrow and the open pile methods ($P>0.05$; 0.118). N= number of samples. S.D= Standard deviation.

Table 8. The Weekly Mean pH Values for the Various Waste Composted.

Week	MEAN VALUE		
	Cow	Poultry	Municipal solid Waste
Week 1	8.050	6.967	8.067
Week 2	8.275	7.025	8.508
Week 3	8.425	7.275	8.675
Week 4	8.833	7.592	8.758
Week 5	8.800	7.808	8.975
Week 6	8.567	7.733	8.667
Week 7	8.300	7.500	8.325
Week 8	8.275	7.442	8.217
Week 9	8.175	7.392	8.058
Week 10	8.133	7.442	8.017
Week 11	8.008	7.425	7.917
Week12	8.000	7.367	7.883

The pH of the various waste types composted were significant difference between the various waste composted (P< 0.05; 0.000).

Table 9. The Mean Conductivity (μs/cm) Values for the Composting Techniques Used

LOCATION	Windrow			Open pile		
	Mean	±	S. D	Mean	±	S. D
Week1	1,158.333[a]		299.5	1,158.333[a]		229.5
Week2	1,030.000[a]		267.6	927.778[b]		347.2
Week3	1,013.889[a]		270.6	853.333[c]		361.0
Week4	1,046.667[a]		239.0	898.889[c]		393.3
Week5	961.667[b]		356.4	876.667[c]		360.0
Week6	791.667[b]		396.4	795.000[d]		403.6
Week7	824.444[b]		394.4	756.111[d]		425.7
Week8	820.556[c]		405.7	796.111[d]		439.1
Week9	869.444[c]		415.5	735.556[d]		435.2
Week10	805.000[c]		409.2	726.667[d]		436.4
Week11	801.111[c]		412.0	723.889[d]		434.2
Week12	801.111[c]		411.5	716.667[d]		434.1

Mean with different superscript are significantly different ($P<0.05$) using Student t- test and Duncan's multiple range test. There was a significant difference between the conductivity values of the windrow and the open pile methods of composting $P<0.05$; 0.000). S.D= Standard deviation.

Table 10. The Weekly Mean Conductivity (µs/cm) Values for the Various Waste Composted.

Week	MEAN VALUE		
	Cow	Poultry	Municipal solid Waste
Week 1	1,073.333	1,316.666	1,085.000
Week 2	862.500	1,319.167	755.000
Week 3	869.167	1,300.00	631.667
Week 4	959.167	1,306.667	652.500
Week 5	839.167	1,300.000	618.333
Week 6	600.000	1,273.333	506.667
Week 7	626.667	1,285.833	458.333
Week 8	610.833	1,295.500	519.167
Week 9	693.333	1,293.333	420.833
Week 10	591.667	1,288.333	417.500
Week 11	578.333	1,290.833	418.333
Week12	574.167	1,286.667	415.833

The results show significant differences between the various waste composted $p<0.05(0.000)$.

Table 11. The Level of Heavy Metals Present in the Various Waste Composted (ppm)

	Lead		Cupper		Nickel		Zinc		Cobalt		Cadmium	
	Conc.	± SD	Conc.	± SD	Conc.	± SD	Conc.	± SD	Conc.	± SD	Conc.	± SD
MSW	0.507	0.0005	0.438	0.0003	0.156	0.0008	15.937	0.0016	0.423	0.0009	0.013	0.0005
PW	0.014	0.0001	0.556	0.0007	0.062	0.0005	5.601	0.0002	0.239	0.0005	0.002	0.0002
CW	-0.038	0.0002	0.201	0.0001	0.048	0.0008	2.054	0.0005	0.297	0.0008	0.001	0.0004

Key: MSW= Municipal Solid Waste, PW= poultry waste, CW= Cow waste.

all the heavy metal tested with Zn having the highest of 15.937ppm concentrations than the composts from poultry and cow waste.

4.2 Microbial Indicator Levels

Many bacteria species were present in the compost (appendix i) out of which *Salmonella typhi, Vibrio cholerae* and *E.coli o157:H7* were carefully selected on the bases of their distribution, spread and virulence of the pathogens. A total of 432 samples, 72 from each sets of composts were collected for both microbiological and physicochemical analysis and helminths monitoring. Comparing the mean counts from the different compost studied the results showed that the mean values for the final compost met the recommended value ($2.0X10^{7}$cfu/g).The total plate count had their mean values greater than 6.0cfu-g (log_{10}), except for compost two (2) which mean values were less than 6.0cfu-g (log_{10}). The coliform counts had their mean values greater than 5.0cfu-g (log_{10}) except for composts 1 and 2 whose mean values were less than 5.0cfu-g (log_{10}). The total mean counts for the total plate count were 6.30cfu-g (log_{10}) and 5.05 cfu-g (log_{10}) for the total coliform counts. In comparing the total pate counts and the total coliform counts, there is a significant reduction in counts between the weeks ($P<0.05$) Table 12).

The effect of composting on the counts (weekly), showed a general decline in the total viable and total coliform count. The total plate count declined from week 1 to 5, it remained at >5.0cfu-g (log_{10}) from week 6 to 9 and week 11 to 12 with their weekly counts remaining significantly different. The total coliform counts decline steadily from week 1 to 9 and week 10 to 12 remained > 2.0 cfu-g (log_{10}) to the end and their weekly counts was significantly different. The rate of decline showed no significant difference between the total plate count and the total coliform count ($P>0.05$; 0.387) (Tables 13).

Table 12. Mean of the Total Plate and Counts of the Compost (log10 cfu-g)

		N	MEAN ±	S. D
Total Plate Count	COMPOST1	72	6.01^{a}	1.454
	COMPOST2	72	5.91^{ab}	1.429
	COMPOST3	72	6.40^{ab}	1.462
	COMPOST4	72	6.81^{ab}	1.539
	COMPOST5	72	6.51^{c}	1.661
	COMPOST6	72	6.16^{d}	1.489
	Total	432	6.30	1.530
Total Coliform Count	COMPOST1	72	4.57^{a}	2.583
	COMPOST2	72	4.60^{b}	2.441
	COMPOST3	72	5.24^{b}	2.694
	COMPOST4	72	5.27^{b}	2.190
	COMPOST5	72	5.62^{b}	2.399
	COMPOST6	72	5.00^{c}	2.034
	Total	432	5.05	2.416

Means with different superscript are significantly different ($p<0.05$) using ANOVA and Duncan's multiple range test. N= number of samples. S.D= Standard deviation.

KEY: Compost 1= open pile cow waste

Compost 2= windrow cow waste

Compost 3= open pile poultry waste

Compost 4= windrow cow waste

Compost 5= open pile Municipal solid waste

Compost 6= windrow municipal solid waste.

Table 13. Mean and Standard Deviation of Compost for each Week Counts (log10 cfu-g)

Week		Total plate count		Total coliform count	
	N	Mean ±	S. D	Mean ±	S. D
Week1	72	9.43[a]	0.058	9.41[a]	0.055
Week2	72	8.44[ab]	0.592	8.26b[c]	0.563
Week3	72	7.57[ab]	0.610	7.25[c]	0.740
Week4	72	6.83[ab]	0.825	6.08[d]	0.759
Week5	72	6.54[c]	0.803	5.44[e]	1.330
Week6	72	5.89[d]	0.581	5.14[f]	0.829
Week7	72	5.55[e]	0.481	4.31[f]	1.267
Week8	72	5.20[f]	0.550	3.81[g]	0.854
Week9	72	5.08[g]	0.558	3.14[h]	0.856
Week10	72	4.86[h]	0.179	2.86[i]	0.894
Week11	72	5.11[i]	0.179	2.47[j]	1.234
Week12	72	5.08[j]	0.324	2.44[k]	1.219
Total	864	6.30	1.530	5.05	2.416

Mean with different superscript are significantly different ($p<0.05$) using Student t-test and Duncan's multiple range test (In between groups within the groups) but show no significant differences between the total plate count and total coliform count $P>0.05$ (0.387). N= number of samples. S.D= Standard deviation.

The weekly mean total plate counts for the various waste types composted showed steady decline which stabilizes at week 11 and 12. However between week 6 and 8 cow waste had their counts >5.0 cfu-g ($\log_{10}$), poultry waste had their counts above 5.0 cfu-g ($\log_{10}$) between week 6 and 9 while municipal solid waste had their count above 5.0 cfu-g ($\log_{10}$) between week 7 and 9. The results showed no significant difference between the waste types $p>0.05$ (0.418, 0.174 respectively) (Table 14).

The results for the two types of composting techniques used also revealed a steady decline in the mean counts. the counts had a steady decline from week 1 to 5 and stabilizes at week 6 to 9 at counts above 5.0 cfu-g ($\log_{10}$) although their differences was statistically significant. The last 3 weeks of composting showed some stability in counts with no significant differences. The results showed no significant difference between the windrow and the open pile compost $P>0.05$(0.151) (Table 15).

The faecal coliform bacteria used as reference indicators were detected in the compost up to fifth (5) weeks and the average total coliform counts had a steady decline from week 1 to 8. Week 4, 5 (windrow) and 5, 6 (open pile) counts were above 5.0 cfu-g ($\log_{10}$) and had significant differences between counts. The last three (3) weeks had stable counts (>2.0 cfu-g) with no significant differences between their values. The results showed a significant differences between the windrow and the open pile method used $P<0.05$; 0.026) (Table 16).

There was generally decline in total coliform count for all the waste types composted from week 1 to 9 and stabilizes at week 10 to 12 for the waste types except for municipal solid waste. The municipal solid waste decline followed a different pattern, between week 5 and 6 counts stabilizes at > 5.0 cfu-g, between week 7 and 8, counts stabilizes at >4.0

Table 14. The Weekly Mean Total Plate Counts for the Various Waste Composted (log10 cfu-g)

Week	MEAN VALUE		
	Cow	Poultry	Municipal solid Waste
Week 1	9.412	9.437	9.428
Week 2	8.628	8.245	8.449
Week 3	7.732	7.397	7.592
Week 4	6.804	6.603	7.091
Week 5	6.464	6.324	6.829
Week 6	5.833	5.653	6.172
Week 7	5.536	5.507	5.607
Week 8	5.047	5.189	5.367
Week 9	4.951	5.049	5.237
Week 10	4.843	4.767	4.973
Week 11	5.079	5.048	5.198
Week12	5.000	5.075	5.174

The rate of decline showed a significant weekly difference among the various waste types ($P<0.05$, 0.001) but showed no significant difference between the different waste types $p>0.05$ (0.418, 0.174) respectively.

Table 15. The Total Plate Count for the Two Composting Techniques used for 12 Weeks (log10 cfu-g)

Location	Windrow		Open pile	
	Mean	± S. D	Mean	± S. D
Week1	9.426^{a}	0.059	9.426^{a}	0.059
Week2	8.450^{b}	0.612	8.431^{b}	0.590
Week3	7.539^{c}	0.613	7.608^{c}	0.622
Week4	6.703^{d}	1.007	6.962^{d}	0.593
Week5	6.521^{e}	0.832	6.557^{e}	0.797
Week6	5.763^{f}	0.663	6.009^{f}	0.473
Week7	5.461^{g}	0.501	5.639^{g}	0.456
Week8	5.202^{h}	0.580	5.201^{h}	0.536
Week9	5.046^{i}	0.594	5.112^{i}	0.535
Week10	4.816^{i}	0.212	4.907^{i}	0.202
Week11	5.071^{i}	0.185	5.146^{i}	0.169
Week12	5.128^{i}	0.425	5.038^{i}	0.177

Means with different superscript are significantly different ($P<0.05$) using student t- test and Duncan's multiple range test. The total plate count showed no significant difference between the windrow and the open pile compost $P>0.05(0.151)$. N= number of samples. S.D= Standard deviation.

Table 16. The Total Coliform Count for the Various Composting Techniques Used (log10 cfu-g)

LOCATION	Windrow			Open pile		
	Mean	±	S. D	Mean	±	S.D
Week1	9.409^{a}		0.056	9.409^{a}		0.056
Week2	8.226^{b}		0.577	8.300^{b}		0.563
Week3	7.179^{c}		0.747	7.322^{c}		0.748
Week4	5.899^{d}		0.829	6.252^{d}		0.656
Week5	5.207^{e}		1.644	5.674^{e}		0.909
Week6	4.941^{e}		0.403	5.333^{f}		1.082
Week7	4.182^{f}		1.646	4.431^{g}		0.749
Week8	3.878^{g}		1.424	3.749^{h}		1.078
Week9	2.913^{g}		1.109	3.370^{i}		0.411
Week10	2.793^{h}		1.039	2.917^{j}		0.748
Week11	2.217^{h}		1.419	2.717^{j}		0.995
Week12	2.500^{h}		1.150	2.383^{j}		1.316

Means with different superscript are significantly different ($p<0.05$) using student t- test and Duncan's multiple range test. It also showed a significant differences between the windrow and the open pile method used ($P<0.05$; 0.026).
S.D= Standard deviation.

cfu-g, between week 9 and 10, counts stabilizes at 3.0 cfu-g and between week 11 and 12 counts were > 2.0cfu-g ($\log_{10}$). There was no significant differences between the waste types composted (P>0.05; 0.340, 0.587); however there was a stable decline in microbial count with the age of the compost for all the waste types (Table 17).

The *Salmonella typhi* and *Vibrio cholerae* colonies were not detected by the fourth (4) week of composting. Feacal coliform were completely eliminated by the sixth (6) week of composting, no re-growth was further noticed throughout the remaining weeks of composting (Table 18). Table 19 shows the frequency of occurrence of target pathogenic microorganism, their characterization and their distribution in the two composting techniques used. A total of 109 suspected *Vibrio*, 152 *E.coli*, and 105 *Salmonella* isolates were tested using biochemical and serological test (Plate 1and 3). Out of these number 97(88.9%) *Vibrio* isolates were confirmed to be *Vibrio cholerae,* 140 (92.1%) were *E.coli 0157,* and 98 (93.3%) were *Salmonella typhi.* From these positive cases, 45 (46.4%) *Vibrio cholerae*, 64 (45.7%) *E.coli* 0157 and 47(48%) *Salmonella typhi* were from the open pile methods while 30 (30.9%) *Vibrio cholerae,* 76(54.3%) *E.coli* 0157 and 51(52%) *Salmonella typhi* were from the windrow method of composting.

In all the waste types composted, *Ascaris lumbricoides* had the highest occurrence in the first and second week after which they were completely eliminated in all the waste types in the compost technique used. For the municipal solid waste, the numbers of *A. lumbricoides* eggs found in the first week were reduced to less than half and other helminths were reduced significantly, hook worm were completely eliminated beyond detection levels in week two of composting in both windrow and the open pile methods (Table 20).

Table 17. The Weekly Mean Total Coliform Counts for the Various Waste Composted (log10 cfu-g)

Week	MEAN VALUE		
	Cow	Poultry	Municipal solid Waste
Week 1	9.382	9.433	9.412
Week 2	8.435	8.258	8.097
Week 3	7.373	7.208	7.171
Week 4	6.096	5.815	6.316
Week 5	5.575	5.057	5.690
Week 6	5.338	4.933	5.139
Week 7	4.081	4.330	4.508
Week 8	3.829	3.591	4.021
Week 9	3.013	3.388	3.023
Week 10	2.550	2.840	3.175
Week 11	2.525	2.600	2.275
Week12	2.800	2.000	2.525

The rate of decline showed a significant weekly difference among the various waste types ($P<0.05$; 0.001) but showed no significant differences between the waste types composted $P>0.05$; 0.340, 0.587),

Table 18. Occurrence of Salmonella/Vibrio spp and Feacal Coliform for the Various samples

Week	*Salmonella typhi*	*Vibrio cholerae*	*E.coli O157:H7*
1	+	+	+
2	+	+	+
3	+	+	+
4	–	–	+
5	–	–	+
6>above	–	–	–

Table 19. Characterization and Distribution of Isolates from the Different Compost.

TYPES OF ISOLATES	No. of isolates tested.	Total no. of positive isolates	Sources and Number of Isolates	
			Open pile	windrow
Vibrio spp	109	97	54	43
Vibrio cholerae	97	75	45	30
Inaba	-	51	39	12
Ogawa	-	24	06	18
other Vibrio	12	12	09	3
E.coli	152	140	64	76
E.coli 0157	140	78	32	46
Salmonella typhi	105	98	47	51

Table 20. Frequency of Contamination of Municipal Solid Waste Samples with Helminthes

SAMPLE TYPES	No. of samples tested.	No. and % containing pathogens. Windrow								No. and % containing pathogens. Open pile							
		ASC	TRIC	SHIST	FACI	HW Larvae	STRO	HW eggs	Total	ASC	TRIC	SHIST	FACI	HW Larvae	STRO	HW eggs	Total
Week 1	6	24(60)	4 (10)	2(5)	6 (15)	1(2.5)	2 (5)	1(2.5)	40	24(60)	4(10)	2(5)	6 (15)	1(2.5)	2(5)	1(2.5)	40
Week 2	12	11 (73.3)	2 (13.3)	0	1(6.7	1 (6.7)	1 (6.7)	0	16	9 (56.3)	2(13.3)	2(13.3)	1(13.3)	0	1(13.3)	1(13.3)	16
Week 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	*30*	35(62.5)	6(10.7)	2(3.5)	7(12.5)	2(3.5)	3(5.4)	1(1.8)	56	33(58.9)	6(10.9)	4(7.1)	7(12.7)	1(1.8)	3(5.5)	2(2.6)	56

ASC= *Ascaris lumbricoides*, TRIC= *Trichuris trichiura*, SHIST= *Shistosoma haematobium*, FACI= *Fasciola hepatica*, HW =*hookworm*, STRO= *Strongyloides stacularis.*

For the poultry waste, *A. lumbricoides* were reduced by half in the windrow method and slightly higher than half in the open pile method after one week of composting. All the helminths seen during the first week of composting were completely eliminated except for *A. lumbricoides*, *T. trichiura* and hookworm for the windrow method and *A. lumbricoides* and *Shistosoma haemtobium* for the open pile method (Table 21). There were higher numbers of *A. lumbricoides* and *Fasciola hepatica* in the cattle waste at the beginning of composting and after two weeks of composting their numbers were reduced significantly for the two waste types. *A. lumbricoides* were reduced to 4 and 2 for the windrow and open pile methods respectively. *Shistosoma haemtobium* and hook worms disappeared completely after one week of composting from the windrow method while *T. trichiura* and hook worm disappeared after one week of composting in the open pile method of composting (Table 22). The open pile helminths counts were slightly higher than those of the windrow within the first two weeks of composting.

The results for the antibiotic sensitivity response to the various antibiotics were recorded following the measurement of the diameter to the nearest millimeter (mm) from the point of abrupt inhibition of growth using the mm ruler and where there is doubt, the point of 80% inhibition was used as zero edge. The following results were obtained after measured zones were compared with the CLIS (2005) standard guidelines for enterobacteriacaea and *Vibrio sp*. The *E.coli* isolates showed antibiotics susceptibility to Ciproflox (CPX), and showed intermediate susceptibility to peflacine (PEF), Gentamycin (CN), Ampicillin (PN), but they showed resistance to Nalidixic acid (NA), Septrin (STX).

Table 21. Frequency of Contamination of Poultry Waste Samples with Helminths

SAMPLE TYPES	No. of samples tested.	No. and % containing pathogens. Windrow								No. and % containing pathogens. Open pile							
		ASC	TRIC	SHIST	FACI	HW Larvae	STRO	HW eggs	Tot al	ASC	TRIC	SHIST	FACI	HW Larvae	STRO	HW eggs	Total
Week 1	6	14(53.9)	8 (30.8)	2(7.7)	0	1(3.9)	0	1(3.9)	26	14(53.9)	8(30.8)	2(7.7)	0	1(3.9)	0	1(3.9)	26
Week 2	12	7 (70)	2 (20)	0	0	1 (10)	0	0	10	8 (88.9)	0	1(11)	0	0	0	0	9
Week 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	*30*	21(58.3)	10(27.8)	2(5.6)	0	2(5.6)	0	1(2.8)	36	22(62.9)	8(22.9)	3(8.6)	0	1(2.9)	0	1(2.9)	35

ASC= *Ascaris lumbricoides*, TRIC= *Trichuris trichiura*, SHIST= *Shistosoma hematobium*, FACI= *Fasciola hepatica*, HW =*hookworm*, STRO= *Strongyloides stacularis.*

Table 22. Frequency of Contamination of Cattle Waste Samples with Helminthes.

SAMPLE TYPES	No. of samples tested.	No. and % containing pathogens. Windrow								No. and % containing pathogens. Open pile							
		ASC	TRIC	SHIST	FACI	HW Larvae	STRO	HW eggs	To tal	ASC	TRIC	SHIST	FACI	HW Larvae	STRO	HW eggs	Total
Week 1	6	17(30.9)	11 (20)	2(3.6)	16(29.1)	1(1.8)	5(9.1)	3(5.5)	55	17(30.9)	11(20)	2(3.6)	16(29.1)	1(1.8)	5(9.1)	3(5.5)	55
Week 2	12	4 (40)	1(10)	0	3(30)	1 (10)	1(10)	0	10	2 (33.3)	0	1(16.7)	1(16.7)	1(16.7)	1(16.7)	0	6
Week 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	*30*	21(32.3)	12(18.5)	2(3.1)	19(29.2)	2(3.1)	6(9.2)	3(4.6)	65	19(31.1)	11(18)	3(4.9)	17(27.9)	2(3.3)	6(9.8)	3(4.9)	61

ASC= *Ascaris lumbricoides*, TRIC= *Trichuris trichiura*, SHIST= *Shistosoma hematobium*, FACI= *Fasciola hepatica*, HW =*hookworm*, STRO= *Strongyloides stacularis.*

There were combine resistance and susceptibility to Streptomycin (S). The *E.coli* isolates from poultry and municipal solid waste showed resistance to Streptomycin while those from cow waste showed intermediate susceptibility (Table 23). The *Salmonella typhi* isolate showed susceptibility response to Ciproflox, Augmentin, and Gentamycin. There were combine susceptibility and intermediate response to Nalidixic acid, Ampicilin and Streptomycin. The isolates showed resistance response to Septrin and Ceporex (Table 24).

The *Vibrio cholerae* isolate showed susceptibility to Tetracycline, intermediate response to Ciproflox and Augmentin and showed intermidate response to Ampicillin, Gentamycin and Streptomycin. All the isolate irrespective of source showed resistance to Peflacine, Nalidixic acid and Septrin (Table 25).

The results of the PCR bands for the isolates were shown in Figure 1. The PCR banding patterns for the isolates were subjected to further evaluations and the results were shown Fig 3. Dendrogram for cluster analysis of all the isolates were constructed by using NTSYS pc2.0 programme of unweighted pair-group method, arithmetic mean (UPGMA). A binary table or haplotype matrix for each strain were constructed linearly composing presence (1)/absence (0) data derived from the analysis of the gel. Microsoft Excel program was used for putting strains in the rows and markers in the columns. SIMINT program was used to compute similarities or dissimilarities in the form of average taxonomic distance which was used to perform sequential, agglomerative, hierarchical, and nested (SHAN) clustering.

Table 23. Zones of Inhibition for Selected Antibacterial Testing on *E. coli* O157: H7.

	Disc potency (µg)									
Sample	TET (30)	PEF (10)	CPX (10)	AU (30)	CN (10)	SE (30)	CEP (10)	NA (30)	SXT (30)	PN (30)
15m	R	I	S	S	I	R	R	R	R	I
11m	R	I	S	I	S	I	I	R	R	I
14p	R	I	S	I	I	I	R	R	R	I
69p	R	S	S	I	I	I	I	R	R	I
61p	R	I	S	S	I	R	I	R	R	I
70c	R	I	S	I	S	I	I	R	R	I
66c	R	R	S	I	I	I	I	R	R	I
9m	R	I	S	S	I	R	R	R	R	S
30p	R	I	S	I	I	R	R	R	R	I
10c	R	I	S	S	S	I	I	R	R	I
E.coli - ATTCC(25922)	R	I	S	S	S	I	I	I	R	S

KEY: TET= Tetracycline, PEF= Peflacine, CPX= Ciproflox, AU= Augmentin , CN= Gentamycin, SE= Streptomycin, CEP= Ceporex, NA= Nalidixic Acid, SXT= Septrin, PN= Ampicilin .

S= susceptible, I= intermediate R= resistance (S=>18mm, I = 12-17mm and R <11mm) (CLIS, 2005).

Table 24. Zones of Inhibition for Selected Antibacterial Testing on *Salmonella typhi.*

	Disc potency (µg)									
Sample	TET (30)	PEF (10)	CPX (10)	AU (30)	CN (10)	SE (30)	CEP (10)	NA (30)	SXT (30)	PN (30)
14m	I	R	S	S	S	S	R	S	R	S
26m	I	I	S	S	I	I	R	I	R	S
15m	R	I	S	S	S	S	R	R	R	I
34p	R	R	S	I	I	S	R	I	R	I
23p	R	R	S	S	I	S	R	I	R	I
42p	R	I	S	I	I	I	R	I	R	I
20c	I	I	S	S	S	S	R	I	R	I
13c	I	I	S	S	S	S	R	I	R	I
17c	R	R	S	S	I	I	R	I	R	I
15m	I	I	S	S	S	S	R	I	R	I
E.coli - ATTCC(25922)	R	I	S	S	S	I	I	I	R	S

KEY: TET= Tetracycline, PEF= Peflacine, CPX= Ciproflox, AU= Augmentin , CN= Gentamycin, SE= Streptomycin, CEP= Ceporex, NA= Nalidixic Acid, SXT= Septrin, PN= Ampicilin .

S= susceptible, I= intermediate R= resistance (S=>18mm, I = 12-17mm and R <11mm) (CLIS, 2005).

Table 25. Zones of Inhibition for Selected Antibacterial Testing on *Vibrio cholerae*.

	Disc potency (µg)									
Sample	TET (30)	PEF (10)	CPX (10)	AU (30)	CN (10)	SE (30)	CEP (10)	NA (30)	SXT (30)	PN (30)
38m	S	**R**	**I**	**I**	**I**	**I**	R	R	R	**I**
37m	S	**R**	**I**	**I**	**I**	**I**	R	R	R	**I**
40m	S	**R**	**I**	**I**	**I**	**I**	R	R	R	**I**
13p	S	**R**	**S**	**I**	**I**	**I**	R	R	R	**I**
32p	S	**R**	**I**	**I**	**I**	**R**	R	R	R	**I**
36p	S	**R**	**S**	**I**	**I**	**I**	R	R	R	**I**
25c	S	**R**	**I**	**S**	**I**	**I**	R	R	R	**I**
26c	S	**R**	**I**	**R**	**I**	**I**	R	R	R	**I**
38c	S	**R**	**I**	**I**	**I**	**I**	R	R	R	**I**
33c	S	**R**	**I**	**R**	**I**	**I**	R	R	R	**I**
E.coli - ATTCC(25922)	R	**I**	**S**	**S**	**S**	**I**	**I**	**I**	**R**	**S**

KEY: TET= Tetracycline, PEF= Peflacine, CPX= Ciproflox, AU= Augmentin, CN= Gentamycin, SE= Streptomycin, CEP= Ceporex, NA= Nalidixic Acid, SXT= Septrin, PN= Ampicilin.

S= susceptible, I= intermediate R= resistance (S=>18mm, I = 12-17mm and R <11mm) (CLIS, 2005).

Dendogram was constructed from the similarity matrix obtained in the SIMINT program by the unweighted pair group method arithmetic mean (UPGMA). The generated gel banding profile showed rDNA fragments in the isolates. The DNA yielded the bands from 1.5kb (Fig.2) and the genomic finger printings of the organisms are shown in Fig. 4 (dendogram). The sequence of each of these DNA fragment differ between species even though the size of the fragments were the same, the locations of the aforementioned primers annealing sites were different for different species targeted as shown on Fig.3. The sequence of the target isolates, their ascension numbers and the sequence identity for the isolates were shown on Table 26 (Plate 11-17). The sequences however encountered some gaps which were edited and the correct nucleotide sequences were inserted using the sequence analyzer before the blast (Plate 5). These reveal relationships based on 16S rDNA sequence and showed the associated organisms with the 16S rDNA of closely related sequence from other prokaryotes on the phylogenetic tree. The dendogram for *E.coli 0157:H7*, *Salmonella typhi*, and *Vibrio cholerae* has demonstrated a characteristic number of traits as well as separate inter and intra group relationship and produced a comparative overall cluster similarities indices, and genetic relatedness among the isolates.

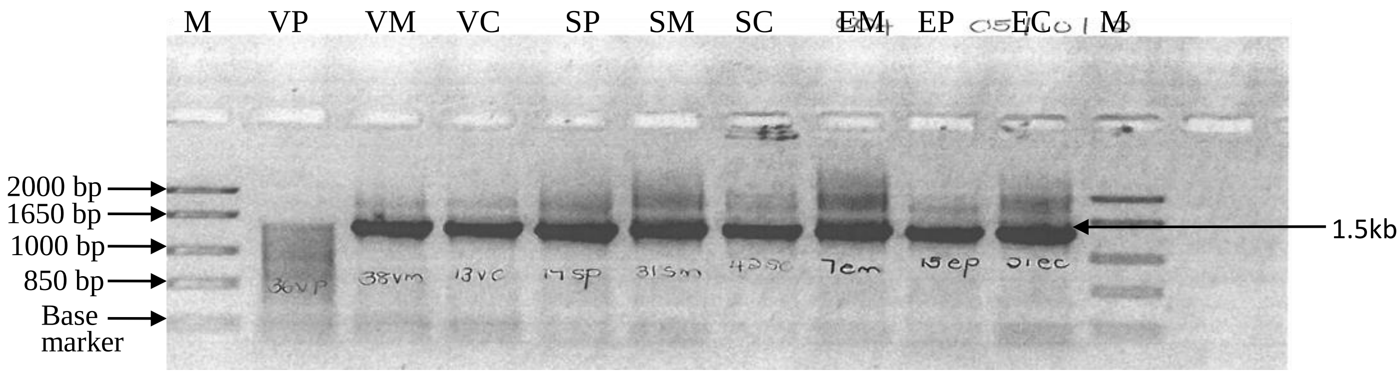

Figure 2: Amplification product using the simultaneous multiple gene detection showing the 1.5kb rDNA bands of *E.coli*, *Vibrio cholerae*, and *Salmonella typhi* from cow, poultry and municipal solid waste.(lane M2kb:2kb plus DNA ladder).

KEY: VP= Vibrio isolates from poultry waste, VM= Vibrio isolates from Municipal solid waste, VC: Vibrio isolates from cow waste, SP= Salmonella isolates from poultry, SM= Salmonella isolates from municipal solid waste, SC= Salmonella isolates from cow waste, EM= *E.coli* from municipal waste, EP= *E.coli* from poultry waste, EC= *E.coli* from cow waste.

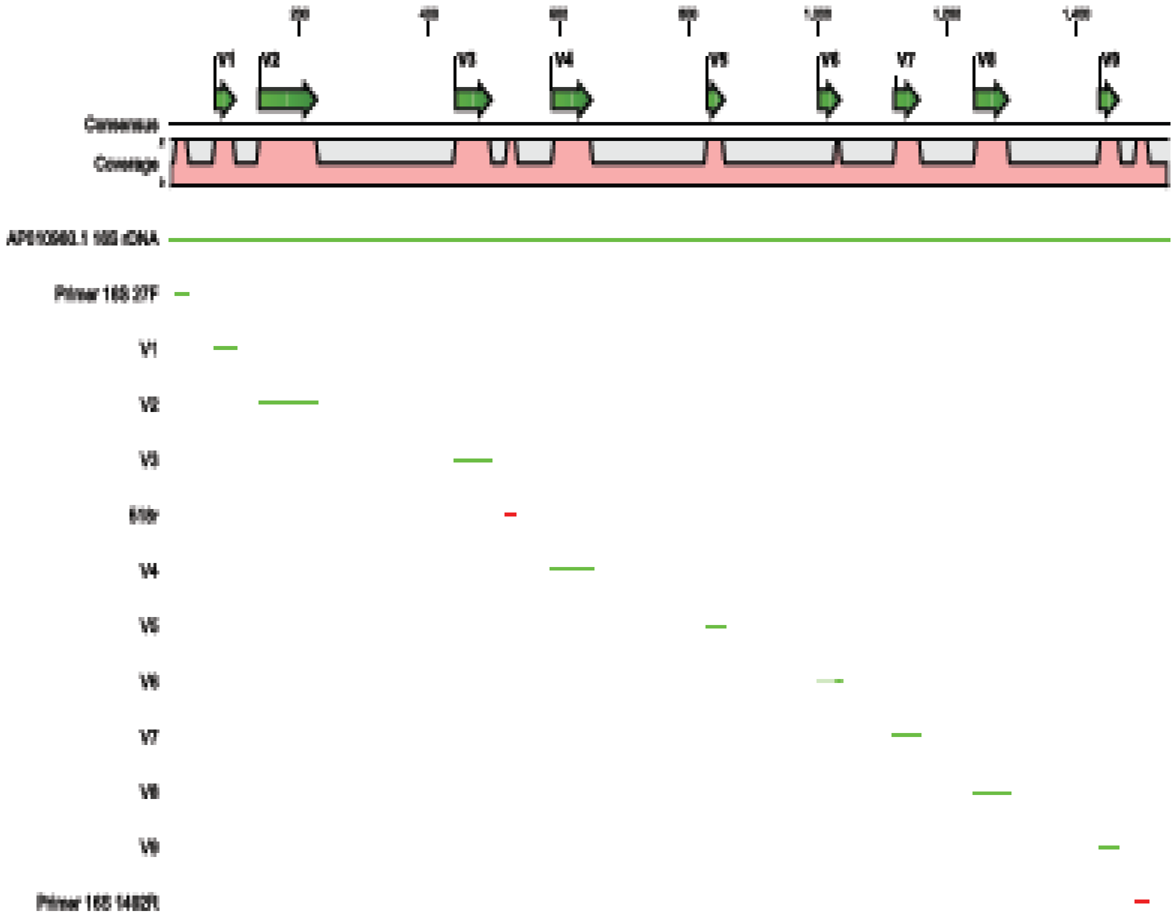

Figure 3: The map of Primers used and Annealing sites in the 16S rDNA Genes for the various isolates.

Table 26: Target Pathogens and their Sequence Profile

Sequence blast	Ascension no.	Sequence identity	Query coverage length	Score bits
>Consensus15EP GGTGGGGTAACGGCTCACCWAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACA CTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG GCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTC AGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCA CCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT GGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGG GAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCG GTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTG ACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA AACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCG ACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG CGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACG GAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGT CAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCC AGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATG ACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAG AGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCT GCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATAC GTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGA	2324	*E.coli 0157:H7*	1412	1174/1172 (100)
>Consensus31SM GCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTRGC ATTCTGATYYACGATTACTAGCGATTCCGACTTCATGSAGTCGAGTTGCAGACTSCAATCCGGAC TACGAYSCACTTTATGAGGTCCGCTTGMTCTCGCGAGGTSGCTTCYCTTTGTAYGCRCCATTGTA GCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCRGT TTRTCACYGGCAGTCTCCTTWGAGTKCCCRSCCKARSYGCTGGCAACWAAGGAYAAGGGTTGC GCTCGTTGCGGGACTTAACCCAACATTTCACRACACGAGCTGACGACAGCCATGCAGCACCTGT STCWSAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTSWGSATGTCAAGRCCAGGTAA GGTTCTTCGCGTTGCWTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC ATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCRATTTAAYGCGTTAGCTSCGGAASC CACACCTCAAGGRCACMACCTCCWAATYGACATCGTTTACGGCGTGGACTACCAGGGTATCTA ATCCTGTTTGCTCCCCACGCTTTCGCACCTSAGCGTCAGTMTYWGTCCAGGGGGYCGCCTTCGC CACCGGTRTTCCTYCMKATMTCTACGCATTTCACCGCTACACCKGRAATTCYACCCCCCTCTACA RGACTCTAGCYTGCCAGTTTYGRATGCAGTTCCCCAGGTTGAGCCCGGGGATTTCACATCCVGA YTTRACRRACCRCCTGCGYGCGCTTTACGCCCAG	1283	*Salmonella typhi*	958	797/866 (92)
>Consensus38VM GTTGCAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCAGT ATGCTGACCTGCGATTACTAGCGATTCCGACTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAA CTGAGAACGGCTTTCTGGGATTGGCTCCACCTCGCGGCTTYGCTGCCCTTTGTACCGTCCATTGT AGCACGTGTGTAGCCCAACTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGG TTTGTCACCGGCAGTCTCCYTAGAGTGCCCAACCAAATGCTGGCAACTAAGGACAAGGGTTGC GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGT CACCCCTGCCCCCGAAGGGGAAGGTACATCTCTGTACCGGTCAGGGGGATGTCAAGAGTTGGT AAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGTCCCCGTCAATT CCTTTGAGTTTCAGCCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTTCAGCA CTGAAGGGCGGAAACCCTCCAACACCTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTAT CTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTATAGGCCAAAGAGTCGCCTTC GCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTTCTCTCC TATACTCAAGCCTCCCAGTTTCCAATGGCCCTCCCCGGTTGAGCCGGGGGCTTCACATCAGACT TAAGAGGCCGCCTGCGCGCGCTT	1687	*Vibrio cholerae* 01	851	855/857 (100)

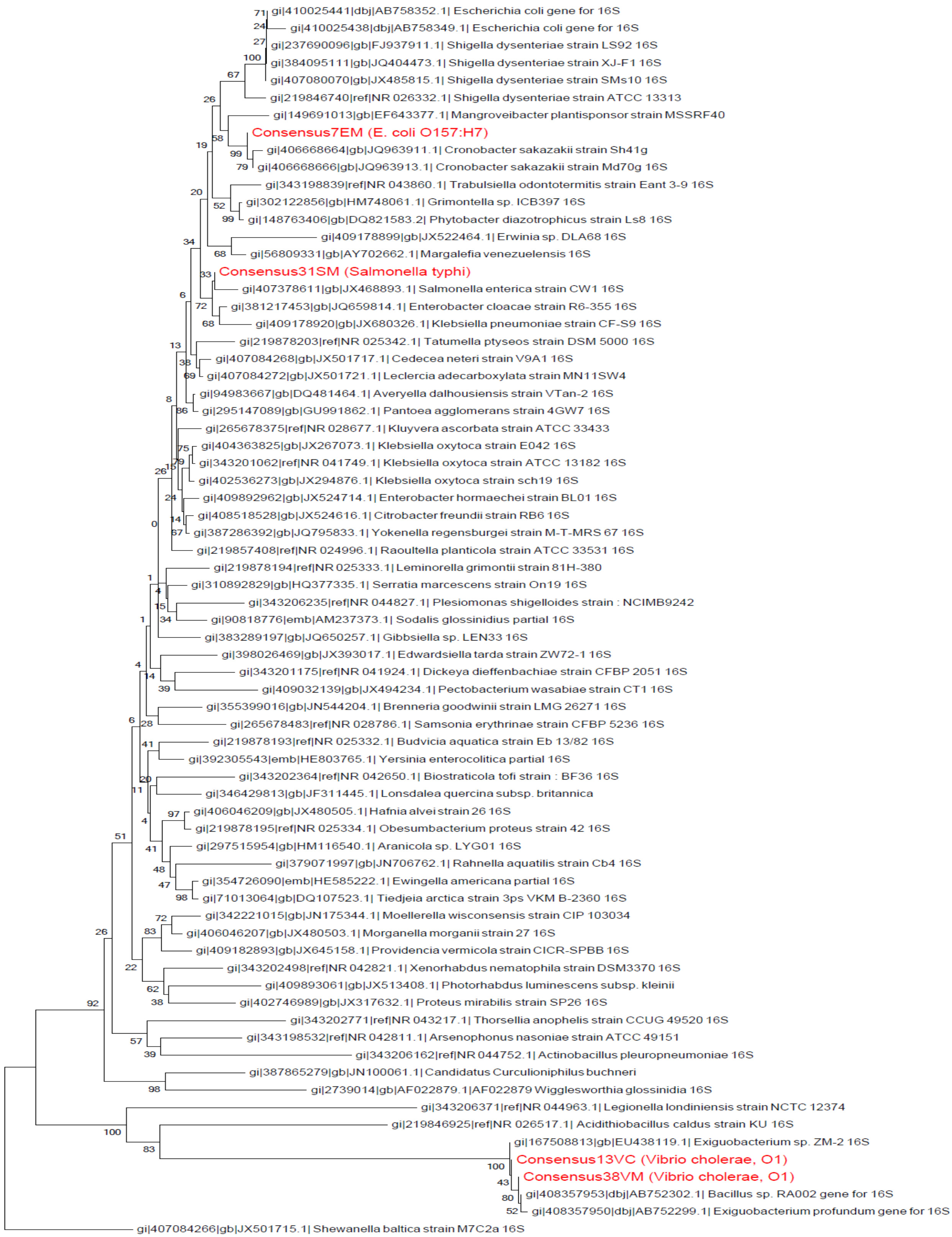

Figure 4: Phylogenetic Relationships Based on the 16S rDNA Sequences Among the Isolates Tested.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Temperature Changes during Composting

Temperature is the main factor that controls microbial activity during composting. Heating is essential to enable the development of a thermophilic population of microorganisms which is capable of degrading the more recalcitrant compounds (natural and anthropogenic), and to kill pathogens (Boulter *et al..*, 2000). Aeration has an indirect effect on temperature by speeding the rate of decomposition and therefore the rate of heat production. The air requirement depends upon the type of waste (type of material, particle size), the temperature of the compost and the stage of the process. Air supply cannot be controlled by the use of a system used for this composting. Under natural conditions warm air diffuses from the top of the windrow drawing fresh air into the base and sides (Hellmann *et al..*, 1997). Aeration is further encouraged by periodic turning of the windrow. Forced aeration has also been used elsewhere successfully on static piles giving a high degree of process control (Sesay *et al..*, 1997), but in this case the natural process of diffusion was used.

The temperature variation (Table 11) was typical of green waste composting. The thermophilic phase, with temperatures above 45^{o}C lasted for a bit over 3 weeks, during which period temperatures as high as 52 ^{o}C was recorded. After six weeks, the temperature dropped dramatically, reaching ambient levels. Thereafter neither compost turning nor moisture correction resulted to any temperature increase.

Microbial activity is influenced strongly by moisture content, activity decreases under dry conditions, and aerobic activity decreases under water-logged conditions due to the resulting decrease in air supply. The recommended optimum water content is 40-60% on a mass basis (Epstein, 1997). There was regular moisturing and turning in this study. Changes in moisture content are related to aeration and temperature, in an aerated static pile system approximately 90% of the heat loss is due to evaporation of water (Sesay *et al.*, 1997). Systems which actively encourage aeration can lead to desiccation and result in a decrease in the rate of decomposition in windrow composting. The compost was kept aerobic to avoid the production of odors. Moisture is essential but if the compost is too wet then anaerobic conditions develop. Anaerobic conditions are also undesirable because of the loss of nitrogen by denitrification. There may also be a buildup of organic acids, such as acetic acid, which can be toxic to plants.

5.1.1 pH Changes During Composting

The pH trend agrees with the findings of (Finstein and Morris, 1975), pH varies within compost and in this study, there was a general trend for the pH which decrease (become more acidic) during the early stages of decomposition and then increased. The initial decrease in pH may be due to the formation of organic acids that are formed during rapid degradation which can occur prior to composting (Hellmann *et al.*, 1997). The subsequent increase in pH may be due to volatilization of organic acids, and the accumulation of ammonia. The optimum pH for decomposition is 6.5-8.5 but in order to avoid excessive ammonia losses in aerated compost systems the pH should be <7.4 (Hoitink and Kuter, 1986). The pH<5 is unfavorable for bacteria, and will prevent a number of important processes including colonization of compost by bacterial biocontrol agents (Hoitink *et al.*, 1997). If a compost pile includes a large amount of bark the low pH of this material (pH 4.5-5.2) should be balanced by the addition of ammonium-containing materials such as poultry manure to increase pH (Hoitink and Kuter, 1986). The final compost product has a pH of between 7.0 and 8.5.

5.1.2 Electrical Conductivity

The fluctuations observed during the composting process could be attributed to the nutrients ions released and the fixation through changes in proliferation of the aerobic microbial population and this varied widely among the different waste composted. The level of conductivity can also be dependent on evaporative potential of the different compost and the salt content of the original composting materials. When composts are applied to soil the electrical conductivity may increase, raising the level of cation-exchange but the soluble salts could also be lowered with rain fall. A higher electrical conductivity may adversely affect the growth of plant because the pH may have been lowered in a short period of time and in a long time excess salts will be washed as leachate during rain. The high amount of organic matter and pH are most important factors that control the availability of micronutrients in the soil (El-Housseini *et al.,* 2002),

5.1.3 Heavy Metals

Heavy metals in composting materials are most times contaminated with potentially toxic heavy metals due to mixture of degradable and non degradable materials. Organic matters often times develop the potentials to absorb these toxic elements when exposed to refuse collections sites or plantations in potentially toxic soil which might impact on the final product of composting. The heavy metals in the municipal solid waste composted had the highest concentrations of heavy metals as compared to the other waste composted in this study, this could be attributed to the availability of metal containing waste at the dump sites. The high level of heavy metals in the solid waste could be attributed to the dumping of plastics, nickel-cadmium batteries, polyvinyl chloride (PVC) and disposal sludge at the dump sites (Jarup, 2003; Ebong, *et al.,* 2008).

The heavy metals from the waste could be a feeding source in the soil and such situation could become unsafe when these metals are eventually taken up by plants and subsequently get into the food chain. Ground water could also be threatened by the metal leaches and could make them unfit for human consumption (Leke, *et al.,* 2011).

In this study heavy metal in the waste composted level was far lower than the standard recommended by the European Union and the USAEPA in the finished products (Table 18). Since compost is designated to be used in the agricultural and horticultural sector, there is considerable concern over the possible heavy metal contamination.

5.2 Microbial Counts during Composting

It is a well known fact that biologically processed organic manures are better than inorganic artificial fertilizers and one of the front liners in the world today is environmental protection and waste management. In addition, the government of Nigeria is encouraging agriculture and local production as against importation. One of the ways to merge these is the conversion of organic wastes to manure for agricultural use. During composting, the microorganisms use the organic matter as a food source, the process produces heat, carbon dioxide, water vapour and humus as a result of growth and activities of microorganisms (Tiquia, 2005). Monitoring of the microbial succession is important in the effective management of the composting process as microorganisms play key roles in the process and the appearance of some microorganisms reflects the quality of maturing compost (Ryckeboer *et al*., 2003).

In this finding, the method of composting did not affect microbial counts ($P>0.05$). Microbial count were significantly ($P<0.05$) affected by duration of composting (Table 1). In the open pile and the windrow techniques used in composting the waste, there was an initial increase in microbial count followed by steady decline. The initial increase could be due to the utilization of nutrients by the microorganisms present (Tiquia, 2005). The decrease in count may be due to the depletion of nutrients in the waste, accumulation of toxic products and unfavorable growth environment (Kowalchuk *et al.,* 1999), and Atkinson *et al.* (1996), stressed that the microbial count should be low and should not contain significant quantities of viable pathogenic organisms.

The microbial count of the different compost groups is illustrated in Table 1 and 2. The indigenous population of total heterotrophic mesophilic bacteria in the fresh material was high for this type of material, possibly due to the gradual collection and stockpiling of the various wastes, which may have allowed some decomposition to begin before composting. The effect of high temperatures exercise on the microbial population is enormous, as high temperatures favor cellulose degradation, bacteria demonstrated a high count at the first few weeks of the study and their numbers declining steadily (Table 1). This decline could be attributed to the fact that cellulose may become inaccessible to enzymatic attack associated with protective substances such as lignin. During composting, pathogens reduction is accomplished to some degree by several processes, including competition between indigenous microorganisms and pathogens, antagonistic relationship between organisms, the action of antibiotics produced by certain fungi and actinomycetes, natural die-off in compost environment, production of toxic bye products such as gaseous ammonia, nutrient depletion and thermal destruction (Hogg *et al.*, 2002).

The pilot laboratory scale data may be insufficient to accurately predict indicators and pathogen survival times in full-scale composting facilities. During full scale windrow and open pile composting, reduction of all organisms took significantly longer time than the pilot scale experiment and *E.coli* reduction took more than 5 weeks (Wichuk and McCartney, 2007)

5.2.1 Bacteria.

This study examines the survival of *Salmonella typhi* during composting, and the temperature did not meet the maximum temperature criterion of 55^{o}C for 15 days for the two composting methods. *Salmonella typhi* level was reduced to below detection limits after 3 weeks for the windrow and the open pile systems. Temperatures were monitored every week at different vertical positions of the compost and were sampled and tested for *Salmonella typhi*, *E.coli 015:H7*, and *Vibrio cholerae*, and by the 4th week temperature climaxed at 45^{o}C, the compost had no detectable *Salmonella typhi*. This result is in agreement with the work of Deportes *et al.* (1998) and Tiquia *et al.* (1998), although temperature were slightly lower in the windrow, *Salmonella typhi* was completely eliminated within 12 days even when temperature were below 55^{o}C.

Both pathogenic and non pathogenic strains of *E.coli* were present in the various waste materials at the beginning of the composting, *E.coli 0157:H7* was detected up to the 5th week of composting and no re-growth was observed after this period. The temperature for the two composting techniques used did not exceed 52^{o}C (Individual temperatures) for 27 days in all the trials, and *E.coli* presence decline steadily. Several studies also examined temperature effect on non pathogenic *E.coli*, all of which demonstrated rapid inactivation even at temperatures were below 55^oC (Pell, 1997; Nicholson *et al*., 2000; Lioyd and Jiewen, 2004).

According to USEPA (1999) standard, the minimum temperature of 55^oC should be maintained for 3 days consecutively, unless the windrow composting is employed. For windrow, a minimum temperature of 55^oC for 15 days consecutively should be maintained with a minimum of 5 turning during the high temperature period.

5.2.2 Helminths.

Helminths ova are of particular concern in compost because they are very resistant to a variety of chemical and physical agents (Haug, 1993) and their eggs pose a significant public health risk. Within two weeks of composting *A. lumbricoides, Trichuri trichura*, hookworm *ova* and all other helminths seen earlier were reduced to non-detectable levels despite the fact that the windrow and the open pile compost from which the samples were collected did not consistently achieve the desired temperature conditions of 55^oC. Similar results were obtained by Strauch (1983), at the end of his composting period where none of the sample tested contained viable helminths eggs and the temperature remained at ambient.

Proper mixing, adequate moisture content and regular temperature distribution may be the possible reason for achieving these helminths free products. Tharalsen and Helle, (1989) reported similar findings that the viable *Ascari eggs* were reduced after two weeks at 37^oC and all eggs were destroyed after 31 days at this temperature. In another similar study by Contreras-Ramos *et al.* (2004), small scale composting of tannery effluent, cow manure and wheat straw took place during winter and the measured temperatures never exceeded 40^oC and viable helminths ova detected initially could not be found in the finished products, however, the author hypothesized the presence of ammonia and the microbial activities within the compost may have been responsible for the destructions of the ova.

5.2.3 Re-growth

Recontamination of the compost pile from external sources and the re-growth from undetectable levels was not experienced in this work probably because all handlers were trained before the experiment started and is one potential explanation for bacterial pathogens not present beyond the high –temperature phase. Re-growth is a problem for certain bacterial pathogens such as *Salmonella typhi* and *E.coli o157:H7* (Jones and Martin, 2003).

5.3 Antibiotic Sensitivity Tests

The high use antibiotics on veterinary prescription in animals of which 10% is used in poultry (Vanden-Bogaard, 1997; Vanden-Bogaard, 2000) are the major factor selecting for antimicrobial resistance in bacteria and additionally crowding and poor sanitation. These three factors are typically interceded in poultry farming and explain the high prevalence and degree of resistance in *E.coli* of poultry in this study (Table 23). Ojoniyi (1985) and Vanden-Bogaard, (1999) found *E.coli* from battery hens to be multi-resistant while non was observed in the free range chicken. During rearing, antibiotics are commonly used and resistance in *E.coli* in avian intestinal tracts may persist for a long time even in the absence of antibiotics (Vanden-Bogaard *et al.,* 1997).

Antimicrobial resistance in pathogenic bacteria of animal and human origin is a major public health issue. Chaslus-Dancla and Martel (1997) recommended that the flouroquinolones should be drug of choice for the treatment of invasive *Salmonella sp* and Mhand *et al.*(1999) concluded that *S.typhimurium* isolate were susceptible to chloramphenicol, tretracycline and quinolones.

Within Salmonella and especially salmonella entrica serotype typhimurium, multi-antibiotic resistance strains are isolated with increased frequency (Botteldoom *et al*., 2004). Because *V. cholerae* can readily exchange genetic information among strains and with other bacterial species, controlling the emergence of resistance requires an understanding of the source of R-plasmids. The gut and other environments (soil, sewage) contain a variety of organisms that cannot be cultured. This makes it impossible to precisely track the transfer and dissemination of resistance genes in nature. Although not definitive, most available data suggest that other enteric bacteria, such as *E. coli* and non-O1, non-O139 *V. cholerae* serogroups in the environment have been the intermediate hosts. Resistance genes are common and plentiful in normal bacteria in the environment, such as *E. coli*. Some of these genes may have first appeared in millions (Lee *et al*., 2003).

There has also been increasing interest in the role that non-O1, non-O139 serogroups may play in the shifting dynamics of *V. cholerae* and its resistance patterns. Since there could be a high rate of genetic exchange among different *Vibrio* strains in the environment, non-O1, non-O139 strains may be important reservoirs of resistance elements. This is especially important since non-O1, non-O139 strains are increasingly resistant to ciprofloxacin and a few other fluoroquinolones, a widely used drugs to which *V. cholerae* O1 remain universally sensitive (Table 24 and 25). Since *E. coli* spend time cycling through animals, humans, sewage, water and soil, one can hypothesize that *E. coli* may have acquired genes that evolved to provide protection against antibiotics produced naturally by soil bacteria. For the antibiotic era, there is environmental selective pressure for the development of resistance (WHO, 2001)

5.4 Polymerase Chain Reaction

PCR amplification with the primers in this case was found to be a fast and reliable method for differentiation and identification of *E.coli,* Salmonella and Vibrio strains. The PCR with primers on repetitive sequences in the DNA can be performed to identify the strains (Jayarao *et al.,* 1993). Chromosomal rDNA from enterobacteriacae contains several 16S rDNA regions and the sequence of each of these fragments differs between species even though the sizes of the fragments are the same, the location of the aforementioned primer annealing sites can be seen on Fig.3 (Lipman *et al.*, 1995). The dendogram for *E.coli 0157:H7, Salmonella typhi,* and *Vibrio cholerae* has demonstrated a characteristic number of traits as well as separate inter and intra group relationship among the organisms that posses these target genes to produced a comparative overall cluster similarities indices, and genetic relatedness among the isolates.

In this study, the DNA finger print of this target isolates from the different waste types was made in order to seek the genotypes existing based on these targeted genes and the identification pattern observed could be used to determine their occurrence and the sequences can be used for finger printing this target strains from environmental samples as well as for epidemiological investigation.

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CHAPTER SIX

6.0 CONCLUSIONS

A wide range of waste can be composted using the windrow and the open pile composting techniques but regulations should place restrictions on the materials that can be used in organic farming and production systems, The pathogenic bacteria in composting animal and municipal solid waste in Zaria were isolated and characterized. There is little research specifically on composting in organic farming and production systems, therefore most of the information is based on studies carried out in conventional farming system. However, composting and the use of composted products, e.g. composted manure, forms a major component of soil fertility management in organic farming systems. A large number of pathogenic bacteria and parasites have access to waste materials including those destined for composting. To cause a hazard they must survive the composting process and be able to gain access to their host, either directly or by contaminating pasture or food crops. Composts may also play a role in maintaining the presence of pathogens in the environment where they may be ingested by food animals. This risk is probably less than that presented by the use of human sewage, sewage sludges and animal wastes in agriculture as fertilizers. The temperature of the composts increased and peaked at 43.9^{o}C and declined steadily to 37.4^{o}C. The pH increased from 7.7 to 8.5 and declined steadily to the end, while conductivity declined steadily from week two to the end. Most pathogens are efficiently removed during the composting of waste and heavy metals were present in all the compost at various degrees.

The phylogenetic trees revealed a high intra and inter relatedness among the consensus and other pathogens. The antibiotic sensitivity pattern demonstrated varied levels of susceptibility and resistance to the antibiotic used with *E.coli* resistant to Nalidixic acid, septrin and tetracycline but susceptible to ciproflox. *Salmonella typhi* showed resistance to septrin, ceporex and susceptible to ciproflox, while *Vibrio cholerae* was resistant to ceporex and peflecine but susceptible to tetracycline.

Animal and human wastes have been used in agriculture as fertilizers and as a method of disposal for thousands of years. Infections in humans and animals because of this practice have only seldom been recorded. The most important agents for humans are those which cause food-borne infections including *Salmonella sp, E. coli O157:H7* and *Vibrio cholerae*.

6.1 Recommendations

Nigerian organic farmers are not using composting to any significant extent at present. Research is needed on the composting system to enable it to be compared with alternative systems developed elsewhere. There is a need to establish more rigorous process models that enable the composting of a variety of materials by a variety of alternative and cheaper methods in order to produce finished compost of a suitable quality for agricultural purposes. A considerable amount of work has been carried out to develop improved methods for preparation and use of compost as fertilizers and to assist crop protection. Most of this work has been done in the United States and much of it by commercial companies. There is an urgent need for research on the efficacy of these products and risk assessments on the colonization of non-aerated compost by human pathogens.

Effective monitoring tools are required to enable different composting systems to be managed to ensure that conditions are such that they will minimize the risk of the production of greenhouse gases (N_2O and CH_4), ammonia, and organic acids. Source segregation of municipal solid waste followed by recycling (for paper, textiles etc) and composting should be encouraged as this gives the lowest net flux of greenhouse gases, compared to other options for the treatment of municipal solid waste. The feedstock materials used for composting may contain a range of potentially toxic elements (PTEs). Upper limits for conventional compost and limits for organic compost derived from household waste should be given in regulations. These limits should take into account the bioavailability of PTEs in compost immediately following incorporation into soil and over time as the compost is decomposed. The waste material used for composting may contain a large number of organic contaminants (pesticides, pharmaceuticals, industrial contaminants). The fate of these compounds during the composting process and their presence in the finished product should be largely determined.

Workshops and seminars be organized and farmers be trained on the production and handling of compost using these two simple techniques requiring minimal cost. This will help achieve the desired food security, increase food productivity, safety, clean and healthy environment.

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Appendix

Plate i. Salmonella colonies on Bismuth sulphite agar.

Plate ii. Composting site showing the two composting techniques.

Plate iii. ***Vibrio cholerae*** **colonies on thiosulphate citrate bile salt (TCBS) agar.**

Plate iv: Microgen reagents used for biochemical reactions tests.

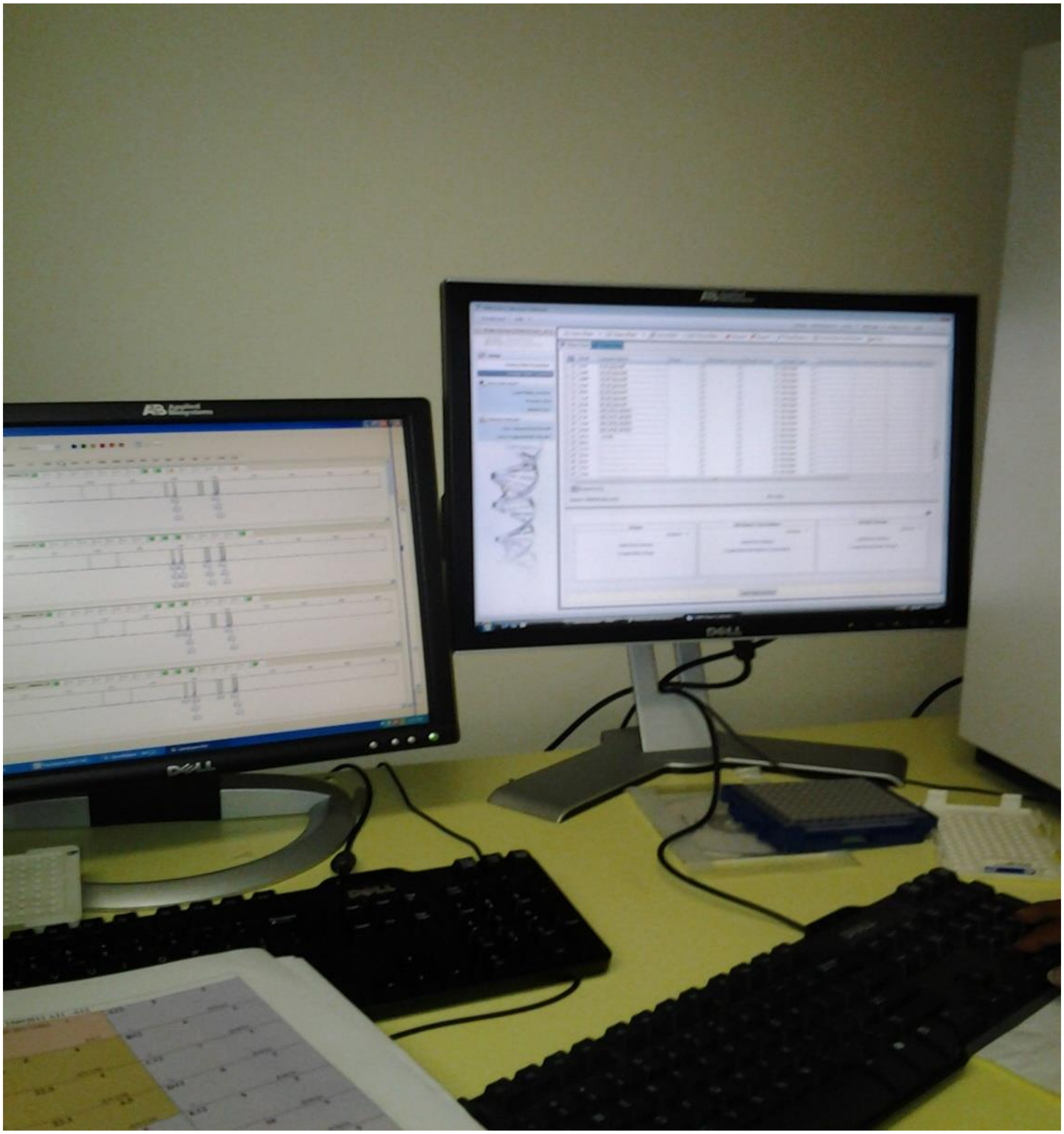

Plate v: Sequencing analyzer.

Plate vi. One of the sampling sites for cattle waste, note that this cattle ranch is located among residential exposing residence to great health risk especially children.

Plate vii. An in house poultry farm, one of the sampling sites for poultry waste.

Plate viii. Children scavenging and animas feeding on refuse dumps exposing them to health hazards, and the animal feeding on the waste could serve as vehicles for disease transmission.

Plate ix. Children and animal on a large waste dump, note that this waste dump sites is located among residential buildings posing great health risk to residence.

Plate x. Children playing Football around waste, exposing them to great health risk. Note that this waste dump sites is located among residential building exposing them to great health risk.

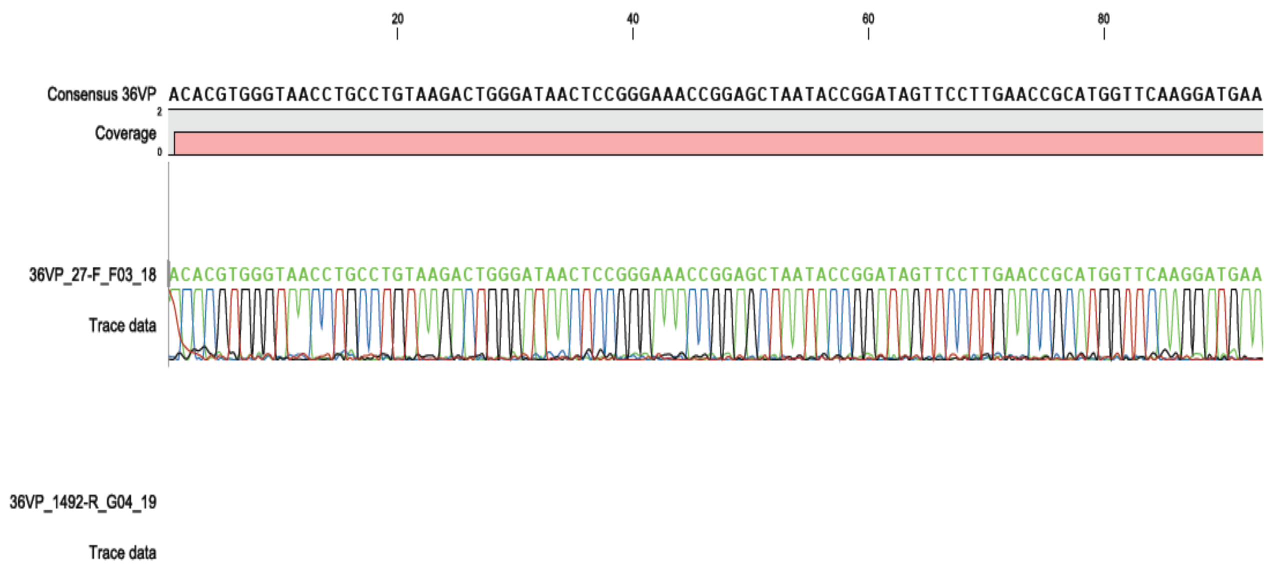

Plate xi: Consesus of Vibrio from poultry waste.

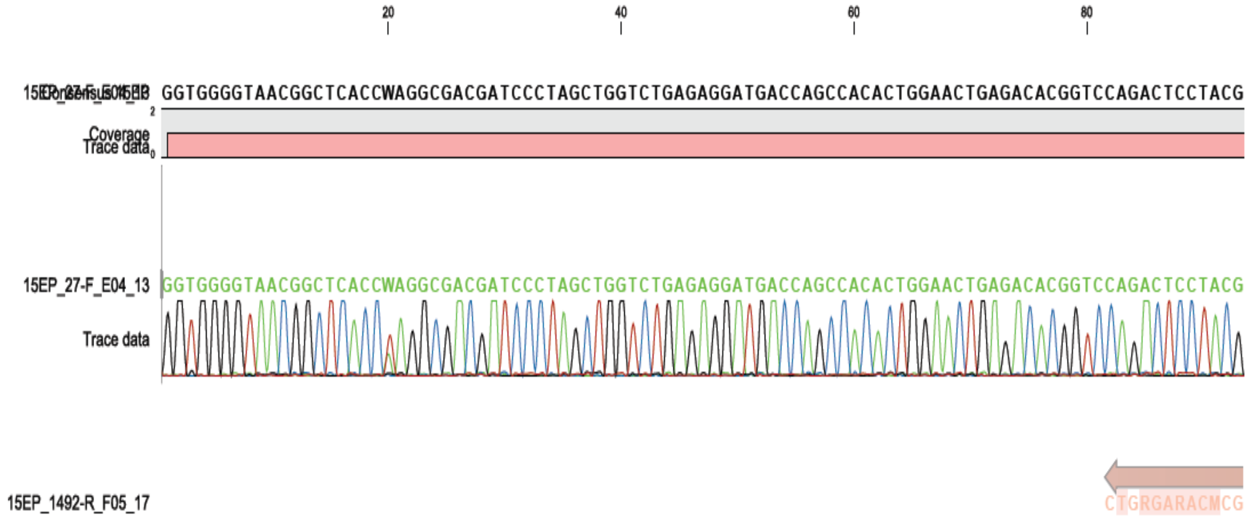

Plate xii: Consensus for *E.coli* from poultry waste

>Consensus7EM

AGCGATTCCGAYTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGSACTTTA
TGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTGTG
TAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATC
ACCGGCAGTCTCCTTTGAGTTCCCGGCCGRACCGCTGGCAACAAARGATAAGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATTTCACAACACRAGCTGACRACAGCCATGCASCACCT
GTCTCAKAGTTCCCGAAGGCACTCCCGCATCTCTGCTGGATTCTCTGGATGTCAAGACCA
GGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC
CGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTSGASTTAACGCG
TTAGCTCCGGAAGCCACGCSTCARRGGCACAACCTCCAAGTMGACATCGTTTACGGCGTG
GACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTT
CGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTA
CACCTGGAATTCTACCCCCCTCTWCKAGACTCAAGCYKRCCAGTTTCAAATGCAGTTCCC
AGGTTGAGMCCGGGGATTTCACATCTGACWTAAYMRACCGCCTGCGTGCGCTTTACGCCC
AGTA

Plate xiii: *E.coli 0157:H7* from municipal solid waste consensus showing the gene length.

>Consensus13VC

TATTGGGCGTAAGCGCGCGCAGGCGGCCTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAA

CCGGGGAGGGCCATTGGAAACTGGGAGGCTTGAGTATAGGAGAGAAGAGTGGAATTCCAC

GTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTTTGG

CCTATAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA

GTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGAAG

CTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGGCTGAAACTCAAAGGAATT

GACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCT

TACCAACTCTTGACATCCCCCTGACCGGTACAGAGATGTACCTTCCCCTTCGGGGGCAGG

GGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG

CAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCATTTGGTTGGGCACTCTARGGAGACTG

CCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGAGTTG

GGCTACACACGTGCTACAATGGACGGTACAAAGGGCAGCRAAGCCGCGAGGTGGAGCCAA

TCCCAGAAAGCCGTTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAA

TCGCTAGTAATCGCAGGTCAGCATACTGCGGTGAATACGTTCCCGGG

Plate xiv: *Vibrio cholerae* from cow waste consensus showing the gene length.

>Consensus31SM

GCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGT

RGCATTCTGATYYACGATTACTAGCGATTCCGACTTCATGSAGTCGAGTTGCAGACTSCA

ATCCGGACTACGAYSCACTTTATGAGGTCCGCTTGMTCTCGCGAGGTSGCTTCYCTTTGT

AYGCRCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCAT

CCCCACCTTCCTCCRGTTTRTCACYGGCAGTCTCCTTWGAGTKCCCRSCCKARSYGCTGG

CAACWAAGGAYAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACRACACGAGC

TGACGACAGCCATGCAGCACCTGTSTCWSAGTTCCCGAAGGCACCAATCCATCTCTGGAA

AGTTCTSWGSATGTCAAGRCCAGGTAAGGTTCTTCGCGTTGCWTCGAATTAAACCACATG

CTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTC

CCCAGGCGGTCRATTTAAYGCGTTAGCTSCGGAASCCACACCTCAAGGRCACMACCTCCW

AATYGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCT

TTCGCACCTSAGCGTCAGTMTYWGTCCAGGGGGYCGCCTTCGCCACCGGTRTTCCTYCMK

ATMTCTACGCATTTCACCGCTACACCKGRAATTCYACCCCCCTCTACARGACTCTAGCYT

GCCAGTTTYGRATGCAGTTCCCCAGGTTGAGCCCGGGGATTTCACATCCVGAYTTRACRR

ACCRCCTGCGYGCGCTTTACGCCCAG

Plate xv: *Salmonella typhi* from municipal solid waste consensus showing the gene length.

>Consensus38VM

GTTGCAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGC

AGTATGCTGACCTGCGATTACTAGCGATTCCGACTTCATGCAGGCGAGTTGCAGCCTGCA

ATCCGAACTGAGAACGGCTTTCTGGGATTGGCTCCACCTCGCGGCTTYGCTGCCCTTTGT

ACCGTCCATTGTAGCACGTGTGTAGCCCAACTCATAAGGGGCATGATGATTTGACGTCAT

CCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCYTAGAGTGCCCAACCAAATGCTGGC

AACTAAGGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCT

GACGACAACCATGCACCACCTGTCACCCCTGCCCCCGAAGGGGAAGGTACATCTCTGTAC

CGGTCAGGGGGATGTCAAGAGTTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACAT

GCTCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGACCGTACT

CCCCAGGCGGAGTGCTTAATGCGTTAGCTTCAGCACTGAAGGGCGGAAACCCTCCAACAC

CTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACG

CTTTCGCGCCTCAGCGTCAGTTATAGGCCAAAGAGTCGCCTTCGCCACTGGTGTTCCTCC

ACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTTCTCTCCTATACTCAAGC

CTCCCAGTTTCCAATGGCCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAG

GCCGCCTGCGCGCGCTT

Plate xvi: ***Vibrio cholerae*** **from municipal solid waste consensus showing the gene length.**

>Consensus15EP

GGTGGGGTAACGGCTCACCWAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCA

CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA

ATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAG

TACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCA

GAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTA

ATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCC

CGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTA

GAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCG

GCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT

ACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGC

TTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCA

AATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCG

AAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGG

GAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAA

GTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAG

GAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTA

CGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGC

AAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAA

GTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGT

ACACACCGCCCGTCACACCATGGGAGTGGGTTGCA

Plate xvii: *E.coli 0157:H7* from poultry waste consensus showing the gene length.

>Consensus17SP

AGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGT

CCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCA

GCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAG

GGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTC

CGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAA

AGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTG

CATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTG

AAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGA

CGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT

AAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAA

GTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCG

CACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTG

ACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGCTG

CATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACC

CTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACT

GGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTG

CTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGC

GTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCG

TGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA

TGGGAGTGGGTTGCA

Plate xviii: *Salmonella typhi* from poultry waste consensus showing the gene length.

>Consensus21EC

TGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAA

CTACTGGAAACGGTAGCTAATACCGCATAACGTCKMMRGACCAAAGWGGGGGACCTTCGG

GCCTCWTGCCATCRGATGTGCCCAGATGGGATTAGCTWGTWGGTGGGGTAACGGCTCACC

TAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGG

TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGC

AGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA

GGSRKTRWGGTTAATAMCYWYRSYSATTGACGTTACCCGCAGAAGAAGCACCGGCTAACT

CCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA

AAGCGCACGCAGGCGGTYTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACT

GCATYTGAWACTGGYMAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT

GAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTG

ACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG

TAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTA

AGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCC

GCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTT

GACATCCASRGAATYYWKCAGAGATGMGRRWGTGCCTTCGGGAACTSTGAGACAGGTGCT

GCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAAC

CCTTATCCTTTGTTGCCAGCGGTYCGGCCGGGAACTCAAAGGAGACTGCCRGTGATAAAC

YGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGT

GCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTG

CGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATC

GTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC

ATGGGAGTGGGT

Plate xvix: *E.coli 0157:H7* from cow waste consensus showing the gene length.

Appendix 1. Biochemical reactions of Bacterial Isolates Obtained during Composting

S/No.	Agar Medium	Colony	Gram Stain	TSI	Simmon citrate	Mortility	Urea	Indole	H_2S	Suspected organism
1	Bismuth sulphite	Black colony	-ve	K/A(+)	+ve	positives	negative	negative	Weak	*S.typhi*
2	✓	White colony	-ve	k/AG-	+ve	positive	negative	negative	-	*S.paratyphi*
3	✓	Black colony	-ve	k/AG+	+ve	positive	negative	negative	strong	*S.heamolyticus*
4	SSA	Pink	-ve	K/A G	-ve	negative	negative	+/-	-	*Shigella sp*
5	EMBA	broown	-ve	A/A G	-ve	-/+	negative	+ve	-	*E.coli sp*
6	SMAC	White	-ve	A/Ag	-ve	+	-ve	+	-	*E.coli o157:H7*
7	EMBA	chocolate	-ve	A/AG	+ve	-ve	+ve	-ve	-	*Klebsiella sp*
8	TCBSA	yellow	-ve curve	A/A	+/_	+/string test	_	+	_	*V. cholerae*
9	TCBSA	Greenish	-ve curve	A/A	+/-	-(string test)	_	+	_	*Vibrio sp*
10	Nutrient agar	white	Rod	Spore	+	+	_	_	-	*Bacillus sp*
11	Nutrient agar	white	+	K/A	-	-	+	-	--	*Actinomycetes sp*

Appendix ii. List of waste dumps sites, cattle ranches and poultry farms located in the study areas.

Waste Types	Location	Types
Municipal solid waste	Angwa karfe	Among households
	Kuspa	"
	Kuspa II	"
	Kuspa no.19	"
	Limanchi no.131	"
	T/ wada/kofar Doka.	"
	Lagos street no.91	"
	Cementary road No.29	"
	Abba Street No.21	"
	Behind Market/Railway	No
	Ontsha Street No69	"
	Muchia Distreet	"
	Kadesoyi	"
	Warri Street.	"
Cattle ranches	Kuspa no.91	Households
	Limanchi corner	"
	Limanchi No.106	"
	Kwaribai No.75	"
	Old Hospital Road	"
	Army Barracks Road 22	"
	King Road 69	"
	Oppo. Main Street/Rail way	Open land
Poultry farms	Aloederi I	Households
	Limanchi no.67	"
	Bakin Kasuwa	Vegetable market
	Bakin Kasuwa II	″
	Kwaribai	Households
	Kwaribai no47	"
	Old Hospital Road	"