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Evaluation of Microbiological Contaminants of Vegetal ProduceCultivated on Animal-Manure-EnrichedSoils

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Abstract:*The study aimed atreconnoitering the bacteriological quality of cabbages (Brassica oleracea), carrots (Daucus carota)and lettuce (Lactuca sativa)cultivated on animal-manure-enrichedsoil in the Kumasi Metropolis of Ghana. Counts of bacteria and fungi (yeast/moulds) were explored using standard microbiological methods. A total number of 78 bacteria isolates were observed, 76.92% of them being Gram-negative. Total bacterial isolates from cabbages, carrots and lettuce respectively, were 30, 21 and 27 with Gram-negative bacteria dominance. The mean microbial counts of 5.82×10^5, 1.03×10^8 and 1.58×10^7 CFU/g for TMC (Total Mesophilic Count), TEC (Total Enterobacteriaceae Count) and TCC (Total Coliform Count) were respectively observed on cabbages whiles the carrots sample had mean counts of 3.55×10^5, 1.86×10^6 and 2.95×10^5 CFU/g for TMC, TEC and TCC respectively. For lettuce, 2.38×10^6, 1.68×10^8 and 1.490×10^8were recorded for TMC, TEC and TCC respectively. The fungal (yeast and moulds) countsyielded 2.16×10^5, 3.74×10^4 and 1.97×10^5 CFU/g for the cabbages, carrots and lettuce respectively. Fusarium semitectum, Aspergillus niger, Rhizopus stolonifer, Aspergillus ochraceus,Mucor spp., and Penicillium expansumwere the detectedspecies isolated. Thus, the selected vegetables investigated were soiled with bacteria populations exceeding acceptable limits of 1.00×10^5 CFU/g whilst the fungal species were within the acceptable limits of 1.00×10^3 CFU/g to $<10^5$ CFU/g.*

Keywords*:Coliform, Enterobacteriaceae, Manure, Mesophilic, Fungi, Vegetables*

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I. Introduction

Vegetal productslike cabbage (*Brassica oleracea*), carrot (*Daucus carota*) and lettuce (*Lactuca sativa*) provide varied essential nutrients mostly micronutrients as documented by George and Pamplona (2005) and Pamplona and Roger (2005), they contribute appreciable amounts of fibreuseful in preventing constipation. Vegetables also contain some vitamins such as provitamin (in red colored vegetables), vitamins B and C (except B_{12}) and vitamin K found in cabbage and spinach. Despite the paybacks,vegetables can be a source of illness such as diarrhea if contaminated with disease causing microorganisms. In Ghana, diarrhea has been reported to be among the top three causes of illnesses (Ababio & Lovatt, 2015). On the international front, diarrheal diseases, due to contaminated and unhygienic foods, are among the leading causes of illnesses and deaths in low-income countries with several outbreaks of disease being attributed to ingestionof contaminated foods, including vegetables (WHO, 2007). There are some reports available demonstrating microbial contamination of vegetables in Ghana (Donkor et al., 2009; Golly et al., 2016; P Mensah et al., 2001; Obeng et al., 2007) resulting in food poisoning.

Unlike food spoilage, for food poisoning, it is rather unfortunate that there is no easy way to express if a particular food is poisoned since the look, smell, and taste of poisoned food is no different from un-poisoned foods (Schmidt, 1999). Generally, vegetables are consumed either raw or minimally processed, therefore, microbial contamination and subsequent consumption of pathogenic bacteria are unavoidable. In the absence of good agricultural and manufacturing practices, post-harvest decontamination practices or appropriate cooking procedure, as it is not possible to conduct a microbial analysis prior to eating, contamination only becomes evident when signs and/or symptoms (nausea, vomiting, abdominal cramps, diarrhea) of illnesses caused by these pathogens are observed. Food borne illnesses such as shigellosis, salmonellosis and listeriosis caused by

Shigella, *Salmonella* and *Listeria monocytogens*respectively, have been identified to be associated with a lot of commodities used for food including raw vegetables(Schmidt, 1999).

In evolvingnations like Ghana, foodborne diseasestriggered by pollutedfresh produce like fruits and vegetables are recurrent and in some expanses they cause a hugepercentage of sickness, nevertheless, owing to the nonexistenceof constant foodborne disease investigations and surveillance in most of these countries most epidemics go undetected and the scientific literature reports only on very few outbreaks(Fleming et al., 2018; Newell et al., 2010; Pennotti et al., 2013). Infections from bacteria are known to be the most predominant infections from eating contaminated vegetables. Most bacteria are harmless, and many of them are enteric in origin, however, pathogenic bacteria which cause non-enteric illness has also been detected in vegetables (Patience Mensah et al., 2002; Okafo et al., 2003). Furthermore, most microbial contamination of fresh vegetables is stated to be associated with improperly composed manures, irrigation water, direct contact with domestic and wild animals, contaminated wash water, human handling, or contamination during packaging, slicing, and food preparation (Beuchat, 2006; Rangel et al., 2005). Naturally, bacteria and fungi form the microflora of plants and herbs which are frequently dominated by aerobic spore-forming bacteria (Cotruvo et al., 2004).

Vegetables are highly perishable therefore, urban and peri-urban agriculture (UPA) engagements ensure that consumers get access to the fresh vegetables with higher nutrient contents due to proximity (Amoah et al., 2007). With an all year-round production, the soils need to be enriched from time to time, toaid continuous use, however, prices of commercial fertilizers are exorbitant and beyond the financial capability of most small-scale farmers. Thus, these vegetable growers use animal manure to enrich the soil in order to fulfill the ever-increasing demand for fresh vegetables. Consequently, reports have shown the increased use of animal manure by farmers engaged in UPA, however, the practice has also engendered public health concerns as animal manures have been identified as sources of pathogenic microorganisms. There is widespread evidence of the rise of outbreaks of diseases in the world implicating the use of manure (animal droppings) as fertilizer in UPA and people consuming contaminated foods (Doyle, 1991; Tood, 1997) including vegetables (Bhaskar et al., 2004; Ghosh et al., 2007; Patience Mensah et al., 2002) with some of the notable organisms being *E. coli 0157:H7*, *Salmonella spp.*, *Shigella* and *S. aureus*(Fleming et al., 2018; Thunberg et al., 2002). In Ghana, it has been documented that, most vegetable farmers in the Kumasi Metropolis use animal droppings as manure for fertilizing their crops as well as using water from polluted sources (Amoah et al., 2007; Golly et al., 2016). Several studies on the microbial contamination of vegetables in Ghana have been documented but little literature exists concerning the microbial contaminants of vegetables cultivated on animal-manure-enriched soils. This study has focused on the potential microbial risk associated with the consumption of vegetable from animal-manure-enrichedsoils in terms of microbiological contaminants especially pathogenic microbes.

II. Materials And Methods

2.1.Study Area

The study samples were obtained from the Gyenyase vegetable growing site, the largest amongst all the peri-urban vegetable growing sites in the Kumasi Metropolis. The cultivated site is estimated at a little over 6 hectares equivalence of 10,000 square meters (2.471 acres) orientated in the moist semi-deciduous forest agro-ecological zone (belt) of Ghana(Amoah et al., 2011). The Gyenyase site supplies the bulk of the vegetables for over the 2,035,064 plus people of the Kumasi Metropolis (Ghana Statistical Service, 2013). Simple agricultural practices such as crop rotation, irrigation from dugout wells, use of animal manure for soil enrichment are under take(Golly et al., 2016) by the growers. The site is also surrounded by other small-scale farmers engaged in cereal grain productions. All experimental procedures were carried out at the Kumasi Center for Collaborative Research in Tropical Medicine, (KCCR), KNUST, Ghana.

2.2.Vegetal Sampling technique

The sampling technique employed was according toGolly et al. (2016) with slight modification.Briefly, only vegetables cultivated at the Gyenyase site were considered in the study. A total of nine (9) samples (three each of cabbage, carrot and lettuce) were collected for their microbial quality assessment in march 2013. By partitioning the site into two equivalent parts with random selection technique, 3 beds were chosen from each half and the vegetables were sampled. With the aid ofsterile sample containers, samples were transported to the laboratory for analysis within 2 h of collection.

2.3.Microbial sample preparation

To ensure aseptic operation procedure, consumables for the microbial investigation were decontaminated under laboratory conditions using standard procedures (Ananthnarayan & Paniker III, 2005; Cheesbrough, 2006). Employing a modified technique by Golly et al. (2016), microbial samples were prepared. Withan aseptic approach, samples weighing 25 g each were agitated in a225 mL bacteriological peptone

waterina sterile stomacher bag for 2 min at moderate speed.Serially dilutedaliquots (10^{-1} to 10^{-6}) were prepared and used in the microbial examinations.

2.4.Microbial enumeration

Microbial examination was done by means of the spread plate technique(Donegan et al., 1991). Yeast and moulds were enumerated on sabouraud dextrose agar (Mast Group Ltd., Mersyside, U.K.) and malt extract agar (Oxiod Ltd. Basingstoke, England) containing 100 mg/L of chloramphenicol (Sigma-Aldrich Co., MO, USA) and 50 mg/L chlortetracycline hydrochloride (ICN Biochemicals Inc, Ohio, USA) to suppress the growth of bacteria and the plates incubated at 25 $^{\circ}$C for 5 to 7 days.Total bacteria were enumerated on plate count agar (Fluka, Sigma-Aldrich Chemie, Switzerland) whereas Coliforms and Enterobacteriaceae were also enumerated on MacConkey agar (Sigma-Aldrich Co., MO, USA) and violet red bile glucose agar (Fluka, Sigma-Aldrich Chemie, Switzerland) respectively at 37 °C for 24 h in aerobic conditions, according toHarrigan and McCance (2014); Balows A (1991). After the appropriate incubation and aided by a colony counter, plates with 30-300 colonies were accepted as valid plates for enumeration. The number of colony-forming units per gram (CFU/g) of the sample was calculated by multiplying the number of colonies counted by the dilution factor.

2.5.Identification and microbiological characterization of isolated microbes

Purification and identification of the microbial cultures were conducted according to Golly et al. (2016) with minor amendments. Briefly,identifiable colonies were repeatedly streaked on appropriate agars and incubated appropriately until pure cultures were obtained. The pure isolates were then identified by examining their colonial,morphological conformationsand Gram reactionsof their cell, and by using standard biochemical test protocols such as catalase as well as coagulase tests. Further identification as well as classification of the microorganisms was accomplished using analytical profile index (API) kits (BioMérieux, SA, France). All isolates were cryo-preserved by mixing the isolated pure colonies with 1 ml of 50% glycerol in cryo-tubes and saved at -80 ^{0}C.

2.5.1.Identification of moulds

Different characteristic features of the isolated fungi were observed and used in their identification employing the fourth edition of the book "Introduction to Foodborne Fungi"(Robert et al., 1995).

2.5.2.Identification of Enterobactericeae

Enterobactericeae were identified per the technique elucidated by Holmes et al. (1978) with modification. Isolates from violet red bile glucose agar (VRBA) (Fluka, Sigma-Aldrich Chemie, Switzerland) were sub-cultured and examined by their colony and cell morphology, oxidase test and Gram reaction. Gram negative rods were further inoculated and the isolates were identified using API 20E kit. In the API analysis, inocula were cultured on nutrient agar (NA) (Mast Group Ltd., Mersyside, U.K.) and incubated at 37 ^{0}C for 24 hours. A single well isolated colony of bacteria on each agar plate was suspended in 5 ml of sterile 0.85% NaCl and homogenized. A 150 µL of the suspension was transferred into the tubes of API 20 E strips that consisted of 20 microtubules, each containing a dehydrated carbohydrate substrate. For the citrate (CIT), Voges Proskauer (VP) and gelatinase (GEL) tests, both the tube and cupules were filled. For the arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), hydrogen sulphide (H_2S) and urase (URE) tests, anaerobiosis was created by overlaying cupules with mineral oil. These were then covered with the incubation lids and incubated at 37 ^{0}C for 18-24 hr. Reagents were added to the tryptophan deaminase (TDA), indole (IND), and Voges Proskauer (VP) tests and the reactions recorded. After the incubation period, the strips were read visually and interpreted using the identification table supplied by the manufacturer.

2.5.3.Identification of Bacillus species

Viableplate count agar (PCA) and nutrient agar (NA)pure sub-cultured colonies were examined by their colony and cell morphology, Gram reaction and catalase test and identified according to a modifiedLogan and Berkeley (1984) protocol. Briefly, isolates that were spore forming rods, Gram positive and catalase positive were suspected to be *Bacillus* species. The API 50 Ch was used in addition to API 50 CHB/E medium (Biomérieux, SA, France) for the identification of *Bacillus* species. In the procedure, about three pure colonies were grown on NA for 48 hours at 30 ^{0}C and dissolved in test tubes containing 2 ml of sterile distilled water. A 150 µL each of the suspensions were pipetted into ampules of the API 50 CHB/E medium and mixed thoroughly. Each suspension was used to inoculate a set of API strips in bubble free tubes. These were then incubated at 30 ^{0}C for 72 hours. Fermented carbohydrates produced a decreased pH which was detected by a change in color from red to yellow. The results were interpreted using the identification table supplied by the manufacturer (Biomérieux, SA, France).

2.5.4.Identification of Staphylococcus species

Bennett et al. (1986) method with modification was applied in the *Staphylococci* identification. Exactly 10 g of sample wasinjected into 90 ml buffered peptone water for revival of metabolically incapacitated bacteria. Upon an overnight incubation, the entire buffered peptone water was pelleted at 4,000 × g for 20 min in a refrigerated (20 ^{0}C) centrifuge (Eppendorf 5810R) and the filtrate decanted. The deposits were streaked on Baird Parkar agar combined with egg yolk for discriminatorysegregation of Gram-positive*Staphylococcus* spp.Gray to jet-black colonies that had opaque zones with outer clear zone were presumptively identified as *Staphylococcus aureus*. The suspected *S. aureus* colonies were transferred into small tubes containing 0.2-0.3 ml Brain-Heart Infusion (BHI) broth and emulsified thoroughly. About 0.5 ml reconstituted coagulase plasma with Ethylenediaminetetraacetic acid (EDTA) was added to the BHI culture and mixed thoroughly and then incubated at 35 ^{0}C for 18-24 hr and examined periodically over a 6 h period for clot formation. Only firm and complete clot that stayed in place when the tube was tilted or inverted was considered positive for *S. aureus*. Partial clotting, formerly 2+ and 3+ coagulase reactions, were tested further and the results confirmed.

2.6.Statistical Analysis

Triplicate experimentalresults were processed using Microsoft Excel 2016. Significant difference amongst means were considered at 95% confidence interval.

2.7.Ethical Clearance

All issues of ethical concerns were fulfilled with the farmers on one part and by acquiring ethical clearance (CHRPE/AP/244/12) from the Committee on Human Research, Publication and Ethics of the Kwame Nkrumah University of Science and Technology, School of Medical Sciences/KomfoAnokye Teaching Hospital.

III. Results and Discussion

3.1.Microbial load on the vegetal samples

Fresh vegetableconsumption has been increasing in recent years and Ghana is not an exception (FAO & World Health Organization, 2005). Fresh vegetables ordinarilyharbornatural, non-pathogenic epiphytic microbes, but during growth, harvest, and from the farm to the fork, the crop could be polluted with pathogens of both animal and human origins. Vegetalproducts frequently are eaten minimally processed, thus, their microbial content may represent a risk factor for the consumer's health and therefore a food safety problem(Brandl, 2006).

The experimentaloutcomesof this study indicated that microbes were abundant on the vegetal exterior, thus all samples investigated were contaminated, representing 100% contamination, but to varying degrees. Total plate count (TPC) of aerobic mesophilic microbes found in food is one of the microbiological indicators of food quality. They reflect the exposure of produce to any form of contamination and generally, the existence of favorable conditions for the multiplication of microorganisms. The bacteria contamination levels recorded in this study for all three vegetables as depicted in **Table 1** were higher than the national reference value of <5.0 $\log_{10}$ CFU/g (Ghana Standard Board, 2003). Cabbage and carrots had mean counts of 5.76 and 5.55 $\log_{10}$ CFU/g, respectively for Total mesophilic counts (TMC). These are relatively higher than 5.13 $\log_{10}$ CFU/g reported byFeglo and Sakyi (2012). Lettuce recorded total mesophilic count of 6.71 $\log_{10}$ CFU/g, a little higher than6.3±0.78 $\log_{10}$ CFU/g reported byPatience Mensah et al. (2002)on street vended salad (including lettuce) in Accra, Ghana. These high mean counts of bacteria could be attributed to the unhygienic practices of farmers (vegetable growers) on the farm such as the use of animal manure for fertilizing the soil as well as the use of ground water (shallow hand dug wells) for irrigation (Amoah et al., 2007; Amponsah-Doku, 2006). Furthermore, no post-harvest reduction action was carried out on the samples prior to reduce the investigations, this may have also accounted for the high contamination levels(Amoah et al., 2007).

Coliforms are usually used as pointers of gastrointestinal tractpollutants from man and animals as well as pointers whose presence will normally designate the plausibleexistence of infectious and disease-causingmicrobes. The mean coliform counts recorded for cabbage and lettuce in this study were above the WHO (Rai & Tripathi, 2007; World Health Organization, 1973) limit of between 3.40 and 6.38 $\log_{10}$ CFU/g for vegetables usually eaten raw but carrot recorded $\log_{10}$ 5.47 CFU/g within the acceptable range (**Table 1**). Total coliform counts (TCC) were 8.17, 7.20 and 5.47 $\log_{10}$ CFU/g for lettuce, cabbage and carrots correspondingly(**Table 1**). The outcome of this study corresponds with similar researches in Kumasi on lettuce (Amoah et al., 2007). In a correlated study on vegetal bacteriological quality in Kano state of Nigeria, high index of bacteria and coliform were exposed(Aliyu et al., 2005).Adebayo-Tayo et al. (2012)on the other hand, reported a relatively lower coliform count. One reason that can be assigned to the high coliform counts in the current study may be the use of contaminated farm irrigation water as observed in previous studies (Amoah et al., 2005; P Mensah et al., 2001). The quality of irrigation water is further compromised by run-off animal

manure deposited on the farms for aging(Amoah et al., 2005; Drechsel et al., 2000; Obiri-Danso et al., 2009). Thus, the high levels of coliform contaminants found in the three vegetables were not unexpected because of the use of the animal manure as a source of nourishment for the vegetables which leads to high contamination (Drechsel et al., 2000; Himathongkham et al., 2000). Drechsel*et al.*, (2000), opined that, fresh poultry litter samples lacking sufficient drying used from time to time for vegetable production in Kumasi, had elevatedfaecal coliform indices between 3.6 x 10^4 and 1.1 x 10^7 CFU/g which is comparable to the results of this research (**Table 1**).

The presence of Enterobacteriaceae is a further indication of faecal contamination of the samples as these microbes are mostly resident in mammalian intestines though they can be found in soil and water. They were the most abundant microbial family recorded in this study compared to the mean total mesophilic and coliform counts (**Table 1**).This finding is consistent with Leff and Fierer (2013)and Heaton and Jones (2008). This phenomenon could be attributed to the human activities around the vegetable farm, such as the use of the animal manure as well as the contaminated irrigation water. Okafo et al. (2003), in a study involving various types of vegetables all irrigated with faecally contaminated water from the same source, found that all the vegetables had a viable count of 100 000 CFU/mL exceeding the regulations of 2000 CFU/100mL (Rai et al., 2007; World Health Organization, 1973). From this research, amongst the three samples, lettuce recorded the highest mean counts for total mesophilic count (TMC), total Enterobactericeae count (TEC) and total coliform count (TCC) except for yeast and moulds count (YMC) (**Table 1**).This finding agrees with Amponsah-Doku (2006)and (Amoah et al., 2005)who stated that much of the surface area of lettuce are exposed to the irrigation water and soil particles from splashes during irrigation by way of overhead technique and this might have accounted for the high contamination.The finding, however, disagrees with other works that states that, cabbages get contaminated the most (Twumasi et al., 2016).

3.2.Identified Bacteria

A sum of seventy-eight (78) bacterial species was secludedin this study.*Pseudomonas aeruginosa* were the most prevalent bacteria isolated (12 out 78) from the three vegetable samples, representing 15.39% (**Table 2**) of the total bacteria isolates. *Escherichia coli* and *Salmonella* spp. each accounted for 14.10% (11 out of 78) of the total bacteria isolates, while *Klebsiella pneumoniae*, also accounted for 10.26% (8 out of 78) prevalence of the total bacteria isolates (**Table 2**).

The isolation of potential pathogenic (**Table 3**) bacteria such as *Escherichia coli*, *Salmonella* spp.as well as *Staphylococcus aureus* is worthy of notification due to their ability to cause infection in humans (Dohmae et al., 2008). *Staphylococcus aureus* is capable of causing human infection such as boils, skin sepsis, toxic shock syndrome, scalded skin syndrome, pneumonia, osteomylitis and food poisoning (Dohmae et al., 2008) and it is also an endotoxin producing bacteria. Most *Escherichia coli* are not pathogenic and are part of the normal human and animal gut flora, however, Enteropathogenic *E. coli* (EPEC) has been associated with outbreaks of gastrointestinal illnesses in nurseries and hospitals and it is also a common cause of infection in children in tropical countries as well as traveler's disease (Dalton et al., 1999).

The dominant or prevalent bacteria species isolated and characterized belonged to the Enterobactericeae group of bacteria specifically *Pseudomonas aeroginosa*, *Escherichia coli*, *Salmonella* spp. as well as *Klebsiella pneumoniae* among others (**Table 2**). This observation agrees withOliveira et al. (2010), who reported a high incidence of Enterobacteriaceae on organically cultivated lettuce than its traditionally cultivated equivalent. In contrast, findings by DuPont (2007) and Leff et al. (2013) had enterobacteria being extra high of about64% on the surfaces of conventionally brandedfresh vegetables (spinach, lettuce, tomatoes, and peaches) when equated with their organically branded equivalent. The presence of these bacteria may be linked to the use of the animal manure in the fertilization of the soil (Mukherjee et al., 2007; Mukherjee et al., 2004) and it has been stated that leafy crops fertilized with inadequately composted manure and those fertilized with animal manure have higher risk of *E. coli* contamination. This is because *E. coli* is a more specific indicator of faecal contamination.

3.3.Identified Moulds

Yeast and moulds are found as part of the normal flora of freshproduce, however, some yeast can produce toxic metabolites, especially mycotoxins which can be resistant to freezing environments and can cause toxicity in the human system as well as off odors and off flavors of foods(Mons, 2004; Respinach al., 2010). A majority of yeasts and moulds occur naturally in the environment (Tournas & Katsoudas, 2005) and may contaminate food during processing, packaging or storage of raw materials or finished products and possibly on the farm during cultivation. The variation in the fungi count in this study may be due to environmental conditions and environmental microflora distribution. In this study(**Table 4**), various genera (*Fusarium*, *Aspergillus*, *Rhizopus*, *Mucor* and *Penicillium*) of moulds were isolated and identified as depicted in **Plate 1**. *Fusarium semitectum*, *Aspergillus niger*, *Rhizopus stolonifer*, and *Aspergillus ochraceus*were observed on the

cabbages while on the carrots,*Fusarium semitectum*, and *Mucor spp.* were found. Lettuce on the other hand had*Mucor spp.*, and *Penicillium expansum* isolated. Their presence may be due to the fact that, these vegetables were cultivated close to the ground and it is a known fact that, moulds are naturally present in the soil (ubiquitous). In this study, the presence of the moulds and their concentrations were within the acceptable limits of between 1.00×10^3 CFU/g to $<10^5$ CFU/g(Abadias et al., 2008) as cabbage, carrots and lettuce recorded 21.6×10^4, 3.74×10^4 and 19.7×10^4 CFU/g respectively (**Table 1**). This finding is similar to that of Abadias*et al.*, (2008), who observed that, whole vegetables generally contain relatively small numbers ($<10^5$ CFU/g) of yeast and moulds. However, Adebayo-Tayo et al. (2012) reported higher values ($1.8\text{-}3.0\times10^6$) for cabbage and carrots in a related study.Geographical difference, difference in laboratory practices could account for this contrast. Though the mould concentrations were close to within acceptable limits (1.0×10^3 CFU/g to $<10^5$ CFU/g) in the current study, their presence is still of public health concern since their presence could result in mycotoxicosis (mycotoxicity: liver cancer, damage tothe immune system, tumors) in humans.

Fusarium contamination of vegetables could be due to field practices (Tournas et al., 2005). Isolation of *Fusarium semitectum* agrees with Nurulhuda et al. (2009)who reported that, *Fusarium semitectum* was the predominant species isolated from six vegetables. With soil and organic substrata as their habitat and widely distributed, *Fusarium* spp. are capable of survival in different environmental conditions because of their quick capacity for transformation, both morphologically and physiologically. The pathogenic *Fusarium* spp. is capable of producing mycotoxins such as Fumonisin/Trichothecenes responsible for diarrheal diseases.They are associated with cereals and can be spread by birds and the wind (Moss, 2008). This may be the reason for their presence since the vegetable site is located in an area where some cultivation of maize and rice are carried out. The presence of *Aspergillus niger* is not surprising since it is widely distributed in nature and is found in soil and litter as well as decaying plant materials. *Aspergillus niger* was among some common fungi isolated from vegetables in some other studies (Akintobi et al., 2011; Al-Hindi et al., 2011).*Penicillium* species are moulds found commonly associated with soil, decaying organic matter, and as storage rots or pathogens of fruits and vegetables. As in this study, Penicillium species have been reported in other studies (Adebayo-Tayo et al., 2012; Dada & Makinde, 2015).Despite their ability to produce antibiotics such as penicillin and griseofulvin, they are also known for their production of toxic metabolites (mycotoxins) such as ochratoxin A, patulin, and penitrem A in food and grains (Andersen & Thrane, 2006). The isolation of *Rhizopus stolonifer*inin this present study agrees with Dada et al. (2015)who isolated *Rhizopus stolonifer including Penicillium spp. and Apergillus spp.* from three leafy vegetables as well as carrots.The isolation of *Rhizopus stolonifer*from the samples in this study further confirmed the studies of Chuku et al. (2008)and Akinmusire (2011), who stated that *Fusarium spp.*, and *Rhizopus stolonifer*were accountable for the soft rot of tomato. Also, Adebayo-Tayo et al. (2012), reported *Rhizopus stolonifer* and *Aspergillus fumigatus* as being responsible for spoilage of cabbage and carrots in storage.

IV. Conclusion And Recommendation

The study revealed thatvegetal products harbouredelevatedlevels of microbial counts. Of particular attention was the presence of pathogenic microbial strains such as *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* which are pointers to contamination from mammalian sources, hence, animal manure stand accused. The implication of the findings of this study presents vegetables from soils enriched with animal-manure as potential vehicles for microbial food poisoning if steps are not taken to reduce contamination levels before eating the vegetables. Engagement of Agricultural Extension Officers to intensify education and assisting these small-scale farmers especially those engaged in urban and peri-urban (UPA) vegetable production to understand and adopt acceptable agricultural practices will go a long way to guarantee continued supply of fresh and healthy vegetables for consumption.

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Table 1: **Microbial counts (CFU/g) of fresh produce at farm level**

Vegetable	TMC	TEC	TCC	YMC
Cabbage	$5.82\times10^{5}\pm0.54^{a}(\log_{10}5.76)$	$1.03\times10^{8}\pm3.09^{a}(\log_{10}8.01)$	$1.58\times10^{7}\pm5.0^{a}(\log_{10}7.20)$	$2.16\times10^{5}\pm0.6^{a}$ ($\log_{10}4.33$)
Carrots	$3.55\times10^{5}\pm0.53^{a}(\log_{10}5.55)$	$1.86\times10^{6}\pm0.06^{a}(\log_{10}6.28)$	$2.95\times10^{5}\pm0.06^{b}(\log_{10}5.47)$	$3.74\times10^{4}\pm0.04^{a}$ ($\log_{10}3.57$)
Lettuce	$2.38\times10^{6}\pm3.0^{b}$ ($\log_{10}6.45$)	$168\times10^{8}\pm8.0^{a}$ ($\log_{10}8.23$)	$1.490\times10^{8}\pm5.0^{a}(\log_{10}8.17)$	$1.97\times10^{5}\pm0.7^{a}(\log_{10}4.29)$

Means that do not share the same superscript in a column are significantly different ($P<0.05$), values in parenthesis are $\log_{10}$. TMC,TEC, TCC and YMC denote Total Mesophilic Count, Total Enterobactericeae Count, Total Coliform Count and Yeast and Mould Counts respectively.

Table 2: Distribution of isolated bacteria from vegetal samples

Bacteria isolated (*N* = 78)	Vegetal sample			Individual Total	Cumulative Total *N* (%)
	Cabbage	Carrot	Lettuce		
Acinetobacter spp.	1(3.3)	1(4.8)		2(2.56)	2(2.56)
Aeromonas salmonicida	1(3.3)	1(4.8)		2(2.56)	4(5.12)
Aeromonas spp.	1(3.3)		2(7.4)	3(3.86)	7(8.98)
*Brevibacillus*spp.	2(6.7)		3(11.1)	5(6.41)	12(15.39)
Brochothrixthermosphacta	1(3.3)			1(1.28)	13(16.67)
Chryseomonasleteola	1(3.3)		1(3.8)	2(2.56)	15(19.23)
Citrobacter spp.			1(3.7)	1(1.28)	16(20.51)
Enterobacter cloacae	2(6.7)	1(4.8)	2(7.4)	5(6.41)	21(26.92)
Enterobacter sakazakii		1(4.8)		1(1.28)	22(28.20)
Erwinia nigrifluens	1(3.3)			1(1.28)	23(29.48)
Escherichia coli	4(13.4)	3(14.3)	4(14.8)	11(14.10)	34(43.58)
Klebsiella pneumoniae	3(10.0)	1(4.8)	4(14.8)	8(10.26)	42(53.84)
*Leuconostocmesenteroides*spp. *cremoris*		1(4.8)		1(1.28)	43(55.12)
Pseudomonas aeruginosa	3(10.0)	6(28.5)	3(11.1)	12(15.39)	55(70.51)
Salmonella spp.	5(16.7)	3(14.2)	3(11.1)	11(14.10)	66(84.61)
Serratia spp.			1(3.7)	1(1.28)	67(85.89)
Staphylococcus aureus		2(9.5)	3(11.1)	5(6.41)	72(92.30)
Staphylococcus spp.	2(6.7)			2(2.56)	74(94.86)
Streptococcus spp.	3(10.0)	1(4.8)		4(5.14)	78(100.00)
Total N (%)	**30(100)**	**21(100)**	**27(100)**		**78(100)**

N denotes number of bacteria isolates and values in parenthesis are percentages

Table 3: Important pathogenic bacteria isolates

Bacteria	Diseases
Escherichia coli	Bloody diarrhea, urinary tract infection
Klebsiella pneumoniae	Destructive change to human lungs
Salmonella spp.	Gastroenteritis or salmonellosis
Staphylococcus aureus	Boils, skin infection, food poisoning, toxic shock syndrome
Staphylococcus spp.	Boils, skin infection, food poisoning, toxic shock syndrome
Streptococcus spp.	Pharyngitis, skin infection

Table 4: **Isolated and identified moulds**

Sample	Moulds
Cabbage	*Fusarium semitectum, Aspergillus niger, Rhizopus stolonifer, Aspergillus ochraceus*
Carrot	*Fusarium semitectum, Mucor* spp.
Lettuce	*Mucor* spp., *Penicillium expansum*

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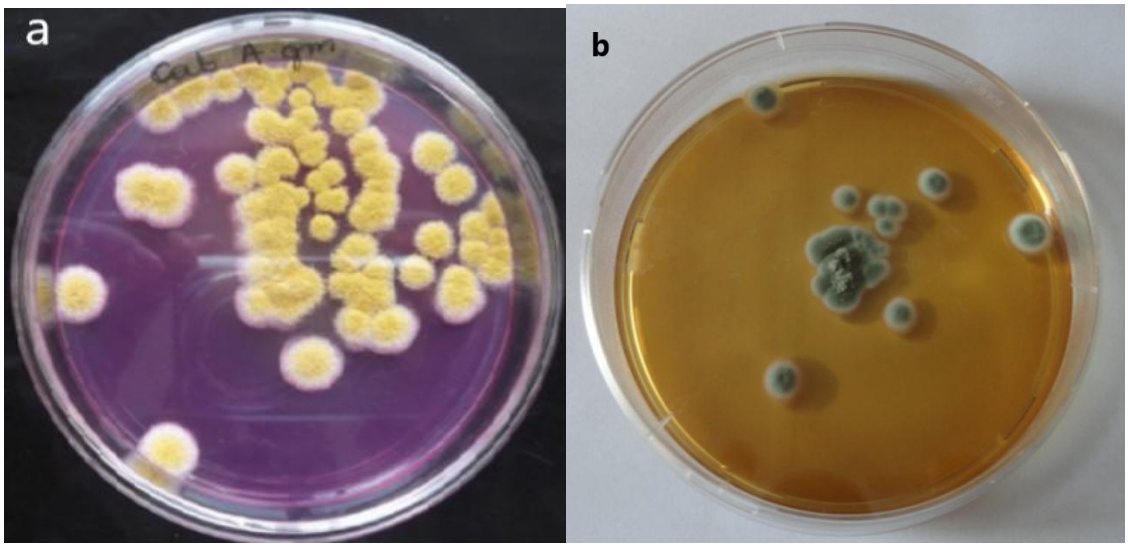

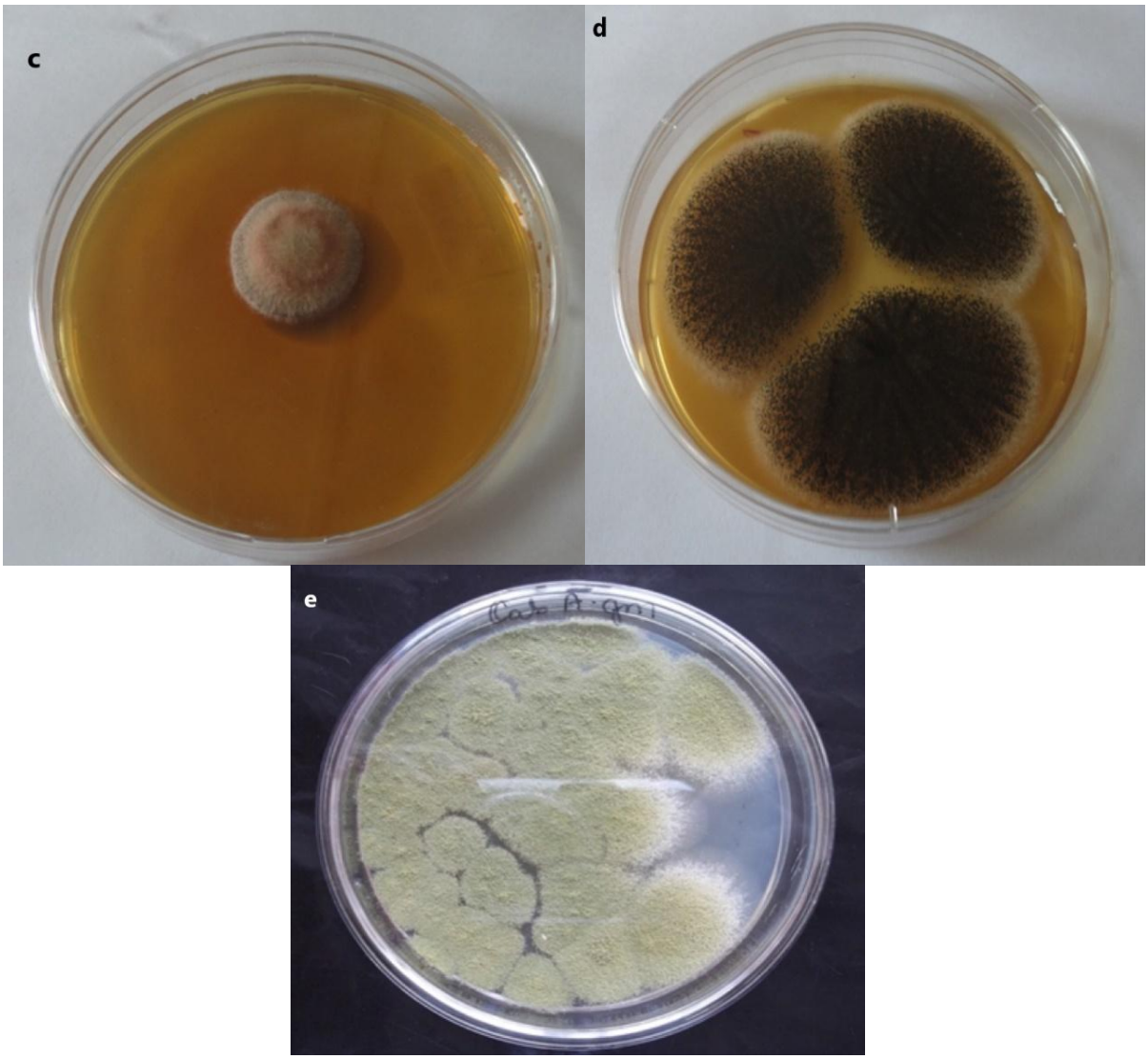

Plate 1: Representative plates of Pure culture isolates of (a) *Apergillusochraceus*, (d) *Aspergillus niger* and (e) *Aspergillus fumigatus*isolated from cabbage as well as (c) *Fusarium semitectum* and (b) *Penicillium expansum*isolated from carrot and lettuce respectively.

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