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Bacteriological Assessment of Some Vegetables and Farm Soils Cultivated With Poultry Manure

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ABSTRACT

Poultry manure is widely used as an organic fertilizer to enhance soil fertility and crop yield, but it poses potential risks of bacterial contamination in vegetables. This study assessed the bacteriological quality of fluted pumpkin (*Telfairia occidentalis*) and waterleaf vegetables cultivated in some poultry-manured farms in Rivers State, Nigeria. Farm soil, vegetable leaves, and poultry manure samples were collected from three (3) farms and from one control farm over a period of three months. Microbiological analysis involved, enumeration and isolation of total heterotrophic bacteria (THB), coliforms, *Salmonella, Shigella, Pseudomonas, Staphylococcus*, and *Vibrio* species using selective media, followed by morphological and biochemical identification of the isolates. Results revealed significantly higher bacterial loads in manured farms compared to the control ($p < 0.05$ for most parameters). Mean THB counts from 2.9±5.9 × 10^6 CFU/g in vegetables from manured soils, with coliform counts up to 8.0×10^6 CFU/g. Identified isolates included *Staphylococcus* spp., *Shigella* spp., *Salmonella* spp., *Bacillus* spp., *Escherichia coli, Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., *Micrococcus* spp., and *Corynebacterium* spp., with *Pseudomonas* and *Bacillus* showing high percentage occurrence of 30%. Distribution varied across samples, highlighting manure as a key contamination source. These findings highlights the public health risks associated with unprocessed poultry manure in vegetable farming, emphasizing the need for proper composting and hygiene practices to mitigate pathogen transmission in food chains.

Keywords: Farm soils, Vegetables, Poultry Manure, Coliforms, Potential Pathogenic Bacteria.

Introduction

Poultry manure (PM) is exceptionally rich in organic carbon, enhancing soil biophysical characteristics and promoting optimal crop performance (Azeez *et al*., 2023). Generally, the application of PM to the soil improves its structure, neutralizes its pH, increases its organic matter content, promotes biological activity in the rhizosphere, decreases erosion, improves infiltration and moisture retention, and increases the soil's nutrient availability (Bello and Olaniyi, 2024). Numerous studies have shown that the application of animal manure significantly affects the microbial communities in a variety of ecosystems, especially in the rhizosphere. For example, an experiment on short-term fertilization was conducted by Moe *et al*., (2019) when demonstrated that the growth of nutrient-rich bacteria is greatly increased by the application of organic fertilizers.

Furthermore, a 33-year fertilization research conducted in arid areas showed that using pig manure greatly increases microbial diversity and abundance (Noosheen *et al*., 2021). These microorganisms can infiltrate plants via several pathways, particularly through organic fertilizers to the soil (Bello and Olaniyi, 2024). The tropical climbing plant *Telfairia occidentalis*, often called the fluted gourd or fluted pumpkin, is indigenous to West Africa and is cultivated for its edible leaves and seeds (Bello and Olaniyi, 2024). The fluted pumpkin, commonly called ugu, especially by the Igbo people of Eastern Nigeria, is a creeping leafy vegetable with large, lobed leaves and a long twisting vine (Ibironke and Owotomo, 2019). The vegetable is grown throughout West Africa's lowland humid tropics and commercially viable with Sierra Leone, Ghana, and Nigeria emerging as the leading producers (Umunnakwe *et al*., 2022).

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The use of soil amendments is the only way to maintain soil fertility and production under a continuous cropping system, which is employed by Nigerian farmers (Azeez *et al*., 2023). Several researchers have recommended and advocated for the integrated use of organic and inorganic fertilizers (Kakar *et al*., 2020). The application of animal manure and other organic material amendments has an impact on the physical properties, fertility, organic matter content, microbiological activity, and amelioration of metal toxicity (Ayeni and Ezeh, 2017; Agbo-Adediran *et al*., 2020). Public health is seriously threatened by the increasing use of poultry manure in vegetable farming, which raises concerns about the spread of bacteria and their genes that are resistant to antibiotics in humans (Adelowo *et al*., 2018). Thus, the present study was undertaken to evaluate the bacteria associated with farms cultivated with poultry manure.

Materials and Methods

Description of Study Area

The four study areas spanned Port Harcourt City Local Government Area, and Obio-Akpor Local Government Area, in Rivers State, Nigeria. Farms A and D are located within the Rivers State University, Nkpolu Oroworukwo, Rivers State, with GPS coordinates of 4.7971°N, 6.9801°E, Farm B is located at the University of Port Harcourt, Choba, Obio-Akpor, Rivers State, with GPS coordinates of 4.869642°N, 6.907772°E, while farm C is located at Eliogbolo community with GPS coordinates of 4.5133°N, 7.0421542°E. The farm lands where the soil samples were collected were farms cultivated with fluted pumpkin and waterleaf, and the soil samples were taken at different points (about 2 meters apart on the farm) to give a composite sample.

Collection of Samples

Four farm soil samples, water leaves, pumpkin leaves and three poultry manure samples (15) were collected from the 4 farms for a period of three months. Leaves of fluted pumpkin (*Telfairia occidentalis*) and waterleaf (*Talinium triangulare*) were harvested from the different farms in the morning hours between 6:00am and 8:00am put into labelled sterile transparent polythene bags and placed in a transparent container.

The samples were transported to the laboratory and analyzed immediately within 6 hours of collection. The soil samples used in this study were collected using hand soil auger at the topsoil at a depths of 0-15 cm at different points 10 meters apart and mixed thoroughly to form a composite sample. The poultry manure sample was also collected aseptically. The samples were kept in a labelled, clean, perforated polythene bag and transported to the Microbiology Laboratory, Rivers State University, for analysis.

Microbiological Analysis

Sample Preparation

The ten-fold serial dilution was carried out. In this method, fresh pumpkin leaf and water leaf samples were rinsed with normal saline to dislodge dirt and make it sterile then placed into a sterile mortar to homogenize. Then 1g of the soil, poultry manure, and pumpkin and water leaf was weighed and transferred aseptically into test tubes containing sterile 9ml diluents (normal saline) which gave an initial dilution of 1:10ml. Subsequent dilutions were carried out by transferring 1ml from the initial dilution to another test tube containing 9ml sterile diluents which gave rise to 1:100 dilution. This process was repeated using each subsequent dilution as a source for the next until a final dilution of 1:1,000,000 ($1:10^{-6}$) was reached (Amadi *et al.,* 2014).

Enumeration of Bacteria in the Various Sample

The total heterotrophic bacterial counts of soil samples were determined using the standard plate count method on nutrient agar. A serial ten-fold dilution was prepared using 1g of soil, poultry manure, pumpkin, and water leaf, and 0.1ml of 10^{-6} dilutions were inoculated and plated in duplicates. Plates were properly labelled and incubated at 37°C for 24 hours.

More so, aliquots (0.1ml) of appropriate dilutions were spread plated in duplicates onto Nutrient, MacConkey, Mannitol salt, *Salmonella shigella,* Thiosulphate citrate bile salt sucrose, Eosin Methylene Blue (EMB) and Cetrimide Agar plates. The plates were incubated at 37°C for 24 hours and 44.5°C for EMB plates (faecal coliform counts). The colonies were counted and described morphologically.
The colonies formed on EMB was used for the enumeration of the population of faecal coliform and

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MacConkey agar for other coliforms while *Salmonella -Shigella* agar for *Salmonella Shigella* counts, Thiosulphate citrate bile salt sucrose for *Vibrio* count, Cetrimide agar for *Pseudomonas* count and Mannitol salt agar for *Staphylococcal* count. Colonies formed on nutrient agar plates were used to estimate the total heterotrophic bacterial counts (THB). Representative discrete colonies were sub-cultured onto freshly prepared sterile nutrient agar plates and incubated at 37°C for 24 hours to obtain pure cultures used for subsequent analysis.

Characterization and Identification of Isolates

The bacterial isolates were identified based on their morphology and biochemical characteristics. Morphological characteristics used were; colony morphology (colour, shape, size, texture and elevation) and Gram reaction. The biochemical tests employed were; methyl red, Voges Proskauer, sugar fermentation, indole, oxidase, catalase, citrate utilization and catalase tests. The tests were carried out as described by Chessbrough, (2005). Isolates were further confirmed using the advanced bacterial identification system (ABIS) online data base (Douglas and Robinson, 2021) and the Bergey's manual of Determinative Bacteriology.

Results

The results for bacterial load are shown in Tables 1, 2, and 3 for months one,two and three respectively. For month one (Table 1), significant difference was recorded for Total Heterotrophic Bacterial (THB) counts in the different farms ($p < 0.001$); a mean value of $2.9 \pm 0.4\times10^5$ CFU/g was recorded in soil without poultry manure, while other mean values ranged from $4.2 \pm 0.7\times10^5$ CFU/g to $4.6 \pm 0.7\times10^5$ CFU/g in soil amended with poultry manure, a mean value of $2.9 \pm 0.6\times10^5$ CFU/g was recorded on pumpkin leaf grown in soil without poultry manure, while mean values ranged from $3.0 \pm 0.3\times10^5$ CFU/g to $5.9 \pm 2.2\times10^5$ CFU/g on pumpkin leaves grown in soil amended with poultry manure, and a mean value of $3.2 \pm 0.5 \times10^5$ CFU/g was recorded for water leaf grown in soil without poultry manure, while mean values ranged from $2.8 \pm 1.1\times10^5$ CFU/g to $4.9 \pm 1.7\times10^5$ CFU/g for water leaf grown in soil amended with poultry manure. Also, significant difference was recorded for Total Coliform counts (TCC) in the different farms ($p < 0.001$); mean values ranged from $2.2 \pm 0.5\times10^5$ CFU/g to $5.1 \pm 2.3\times10^5$ CFU/g in soil amended with poultry manure, while a mean value of $3.7 \pm 0.3\times10^5$ CFU/g was recorded for the control, on pumpkin leaf, a mean value of $6.7 \pm 1.0\times10^5$ CFU/g was recorded for the control, while other mean values ranged between 2.3 ± 0.5 to $6.0 \pm 1.8\times10^5$ CFU/g in soil amended with poultry manure. For Total *Salmonella* counts (TSA), significant difference was recorded in the different farms ($p < 0.001$), and mean values ranged from $6.7 \pm 0.1\times10^3$ CFU/g to $16.7 \pm 0.3\times10^3$ CFU/g in soil amended with poultry manure. For Total *Staphylococcus* counts (TSC), significant difference was recorded for pumpkin leaf in different farms ($p < 0.001$), with mean values ranging from $1.4\pm0.5\times10^5$ CFU/g to $7.3\pm1.9\times10^5$ CFU/g in soil amended with poultry manure, as well as on water leaf with mean values ranging from $1.4\pm0.2\times10^5$ CFU/g to $5.4\pm2.1\times10^5$ CFU/g among others.

Results of the bacterial load for month two is presented in Table 2. Results showed that significant difference was recorded for Total Heterotrophic Bacteria (THB) in the different farms ($p = 0.005$); mean values ranged from $4.1\pm0.5\times10^5$ CFU/g to $4.1\pm0.5\times10^5$ CFU/g in soil amended with poultry manure, $3.1\pm0.4\times10^5$ CFU/g to $4.6\pm0.8\times10^5$ CFU/g on pumpkin leaf grown in soil amended with poultry manure, and $2.7\pm1.3\times10^5$ CFU/g to $4.4\pm1.7\times10^5$ CFU/g on waterleaf grown in soil amended with poultry manure.

Also, significant difference was recorded for Total Coliform counts (TCC) in the different farms ($p < 0.001$); mean values ranged from $2.2\pm1.3\times10^4$ CFU/g to $5.7\pm2.5\times10^4$ CFU/g in soil amended with poultry manure, $1.9\pm0.06\times10^4$ CFU/g to $5.9\pm2.3\times10^4$ CFU/g on pumpkin leaf grown in soil amended with poultry manure, and $4.9\pm0.6\times10^4$ CFU/g to $8.0\pm0.8\times10^4$ CFU/g on waterleaf grown in soil amended with poultry manure.

For Total *Salmonella* counts (TSA), significant difference was recorded in the different farms ($p = 0.010$); mean values ranged from $2.6\pm1.6\times10^4$ CFU/g to $3.5\pm3.0\times10^4$ CFU/g in soil amended with poultry manure, and $0.53\pm0.1\times10^4$ CFU/g to $2.2\pm 0.5\times10^4$ CFU/g on waterleaf grown in soil amended with poultry manure.

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Table 1: Bacterial load (CFU/g) of poultry manure, farm soils, and vegetable samples for Month 1

Samples	Bacterial counts (CFU/g) of poultry manure, farm soils, and vegetable samples						
	THB ($\times10^5$)	TC ($\times10^5$)	TS ($\times10^3$)	TSH ($\times10^3$)	TP ($\times10^4$)	TV ($\times10^3$)	TS ($\times10^5$)
PMA	4.4±0.6^{cda}	2.2±0.5^{a}	0.0±0.0	0.0±0.0 ^{a}	0.0±0.0^{a}	0.0±0.0	2.1±0.4^{a}
PMB	4.2±0.7^{bcde}	2.6±1.1^{ab}	6.7±0.1^{ab}	0.0±0.0 ^{a}	0.0±0.0^{a}	0.0±0.0	3.1±0.9 ^{a}
PMC	4.6±0.7^{de}	5.1±2.3^{abcd}	16.7±0.3^{b}	0.0±0.0 ^{a}	0.0±0.0^{a}	0.0±0.0	3.1±1.5 ^{a}
SA	2.8±1.1^{a}	5.1±0.8^{abcd}	6.7±0.1^{ab}	3.3±0.5^{a}	2.0±0.3^{ab}	0.0±0.0	10.4±1.0^{c}
SB	3.7±0.6^{bcde}	7.10±0.8^{cd}	0.0±0.0^{a}	0.0±0.0 ^{a}	0.0±0.0^{a}	0.0±0.0	2.1±0.5 ^{a}
SC	3.9±0.6^{bcde}	5.2±2.8^{abcd}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0^{a}	0.0±0.0	2.9±0.3 ^{a}
SD	2.9±0.4^{a}	3.7±0.3^{abc}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0^{a}	0.0±0.0	2.0±0.3 ^{a}
UA	3.0±0.3^{ab}	6.0±1.8^{bcd}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	2.8±0.3^{b}	0.0±0.0	1.4±0.5 ^{a}
UB	5.9±2.2^{f}	3.5±1.2^{abc}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	2.5±1.9^{b}	0.0±0.0	1.7±0.6 ^{a}
UC	3.9±0.5^{bcde}	2.3±0.5^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	2.4±0.3^{b}	0.0±0.0	2.6±0.5 ^{a}
UD	2.9±0.6^{a}	4.0±1.8^{abc}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	1.1±0.1^{ab}	0.0±0.0	7.3±1.9^{bc}
WA	2.8±1.1^{a}	6.8±0.7^{cd}	16.0±1.5^{b}	27.3±0.3^{b}	0.40±0.6^{a}	0.0±0.0	5.4±2.1^{ab}
WB	4.9±1.7^{ef}	5.5±0.8^{abcd}	3.3±0.8^{a}	17.3±2.7^{b}	0.33±0.5^{a}	0.0±0.0	3.9±0.2^{ab}
WC	3.4±0.6^{abcd}	7.7±0.5^{d}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.17±0.4^{a}	0.0±0.0	2.2±1.8^{ab}
WD	3.2±0.5^{abc}	6.7±1.0^{cd}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	2.0±0.0^{ab}	0.0±0.0	1.4±0.2^{a}
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.000	<0.001

*Means with similar superscript down the group showed no significant differences (P>0.05)

Table 2: Bacterial load (CFU/g) of poultry manure, farm soils, and vegetable samples for Month 2

Sample	Bacterial counts (CFU/g) of poultry manure, farm soils, and vegetable samples						
	THB ($\times10^5$)	TC ($\times10^4$)	TSA ($\times10^4$)	TSH ($\times10^4$)	TP ($\times10^4$)	TV ($\times10^3$)	TSC($\times10^5$)
PMA	4.7±0.4^{e}	2.1±0.6^{ab}	2.9±2.7^{cd}	4.6±1.6^{abc}	3.0±0.4^{ab}	0.0±0.0	1.9±0.1 ^{abc}
PMB	4.1±0.5^{bcde}	2.2±1.3^{ab}	2.6±1.6^{bcd}	1.5±0.7^{a}	2.5±0.3 ^{ab}	0.0±0.0	3.0±1.5 ^{abc}
PMC	4.3±0.5^{cde}	5.7±2.5^{de}	3.5±3.0^{d}	1.5±0.3^{a}	4.7±3.0 ^{ab}	0.0±0.0	3.0±1.8^{abc}
SA	2.9±1.3^{ab}	5.1±1.0^{cde}	1.3±0.7^{abcd}	4.7±2.8^{abc}	3.0±2.4 ^{ab}	0.0±0.0	2.2±0.2 ^{abc}
SB	3.7±0.6^{abcde}	1.6±0.5^{a}	0.97±0.6^{abc}	5.7±1.3^{bc}	20.5±10.1^{d}	0.0±0.0	2.3±0.4 ^{abc}
SC	4.2±0.4^{bcde}	4.2±3.1 ^{abcd}	0.75±0.3 ^{abc}	7.7±0.5^{c}	7.0±0.6^{abc}	0.0±0.0	2.7±0.4 ^{abc}
SD	3.1±0.4 ^{abc}	4.5±4.0 ^{abcd}	0.27±0.2 ^{ab}	5.5±1.6^{bc}	3.2±0.4 ^{ab}	0.0±0.0	1.9±0.3 ^{abc}
UA	3.1±0.4 ^{abc}	5.9±2.3 ^{de}	0.75±0.01^{abc}	4.6±3.8^{abc}	9.2±4.0^{bc}	0.0±0.0	1.6±0.5^{ab}
UB	4.6±0.8^{de}	2.8±0.8^{abc}	0.0±0.0 ^{a}	2.8±2.1 ^{ab}	12.7±10.8^{c}	0.0±0.0	1.4±0.2^{ab}
UC	4.2±0.4^{bcde}	1.9±0.06^{a}	0.0±0.0 ^{a}	3.6±1.7^{ab}	7.0±1.1^{abc}	0.0±0.0	2.7±0.6 ^{abc}
UD	3.2±0.6^{abc}	3.9±2.3^{abcd}	0.0±0.0 ^{a}	2.6±0.8 ^{ab}	2.5±0.6 ^{ab}	0.0±0.0	7.0±2.3^{d}
WA	2.7±1.3^{a}	6.5±0.5 ^{de}	2.2±0.5^{abcd}	1.9±0.2^{a}	2.2±1.3 ^{ab}	0.0±0.0	6.0±2.2^{d}
WB	4.4±1.7^{cde}	4.9±0.6^{bcd}	0.53±0.1 ^{ab}	3.4±1.2 ^{ab}	1.4±0.5 ^{ab}	0.0±0.0	3.9±1.2^{c}
WC	3.3±0.7^{abcd}	8.0±0.8^{e}	1.1±0.2 ^{abc}	3.1±1.9 ^{ab}	0.62±0.07^{a}	0.0±0.0	3.4±0.7^{bc}
WD	3.3±0.5^{abcd}	6.0±0.1 ^{de}	0.55±0.06^{ab}	3.9±2.9^{ab}	3.2±2.5 ^{ab}	0.0±0.0	1.3±0.1^{a}
P-value	0.005	<0.001	0.010	0.002	<0.001	0.000	<0.001

*Means with similar superscript down the group showed no significant differences (P>0.05)

Keys: CFU = Colony forming units; PM = Poultry Manure; S= Soil; U= Ugu (Pumpkin Leaf); W= Water Leaf; ABCD = Stations; THB = Total Heterotrophic Bacteria, TC = Total Coliform, TS = Total *Salmonella*, TSH = Total *Shigella*, TP = Total *Pseudomonas*, TV= Total *Vibrio*,TSC = Total *Staphylococcus*.

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For Total *Shigella* counts (TSH), significant difference ($p = 0.002$) was recorded in soil amended with poultry manure with mean values ranging from $1.5\pm0.7\times10^4$ CFU/g to $4.6 \pm 1.6\times10^4$ CFU/g on pumpkin leaf with mean values ranging from $1.4 \pm 0.5\times10^4$ CFU/g to $7.3 \pm 1.9\times10^4$ CFU/g and on waterleaf with mean values ranging from $1.4 \pm 0.2\times10^4$ CFU/g to $5.4 \pm 2.1\times10^4$ CFU/g among others. For Total *Staphylococcus* counts (TSC), significant difference ($p < 0.001$) was recorded for water leaf in the different farms with mean values ranging from $1.3 \pm 0.1\times10^5$ CFU/g to $6.0 \pm 2.2\times10^5$ CFU/g among others.

For month three (Table 3), significant difference was recorded for Total Heterotrophic Bacteria (THB) in the different farms ($p = 0.023$); mean values ranged between $3.4\pm0.7\times10^5$ CFU/g to $4.6\pm1.6\times10^5$ CFU/g in soil amended with poultry manure, $3.5\pm3.0\times10^5$ CFU/g to $4.3\pm1.2\times10^5$ CFU/g on pumpkin leaf, and $2.3\pm0.5\times10^5$ CFU/g to $3.9\pm1.7\times10^5$ CFU/g on water leaf. Also, no significant difference was recorded for Total Coliform counts (TCC) in the different farms ($p = 0.266$), as mean values ranged from $2.2\pm1.3\times10^4$ CFU/g to $5.7\pm2.5\times10^4$ CFU/g in soil amended with Poultry manure, $1.5\pm 0.5\times10^4$ CFU/g to $4.8 \pm 3.5\times10^4$ CFU/g on pumpkin leaf, and $1.6\pm0.2\times10^4$ CFU/g to $6.7 \pm 0.9\times10^4$ CFU/g on waterleaf. For Total *Salmonella* counts (TSA), no significant difference was recorded for the different farms ($p = 0.394$) as mean values ranged from $2.6\pm0.9\times10^4$ CFU/g to $4.1\pm2.8\times10^4$ CFU/g in soil amended with poultry manure.

The results for bacteria isolated and identified based on morphological and biochemical characterization confirmed the presence of *Staphylococcus Augmentinreus*, *Corynebacterium striatum*, *Shigella* spp., *Salmonella enterica*, *Bacillus* spp., *Micrococcus* sp., *Escherichia coli*, *Enterobacter* sp., *Klebsiella pneumonia* and *Pseudomonas syringae*.

The results for the distribution of bacterial isolates across pumpkin leaf, water leaf, soil sample (control) and poultry manure are shown in Tables 4a, b, c and d, respectively; while percentage occurrence of bacterial isolates across pumpkin leaf, water leaf, soil sample and poultry manure are shown in Figures 1a, b, and c, respectively.

Table 3: Bacterial load (CFU/g) of poultry manure, farm soils, and vegetable samples for Month 3

Sample	Bacterial counts (CFU/g) of poultry manure, farm soils, and vegetable samples						
	THB ($\times10^5$)	TC ($\times10^4$)	TS ($\times10^4$)	TSH ($\times10^4$)	TP ($\times10^4$)	TV ($\times10^3$)	TSC ($\times10^5$)
PMA	4.6 ± 1.6^{d}	6.5 ± 2.9	2.6 ± 0.9	4.9 ± 2.5^{e}	7.5 ± 0.6^{cde}	0.0 ± 0.0	4.0 ± 0.9^{bcd}
PMB	3.9 ± 0.6^{bcd}	3.9 ± 2.3	4.1 ± 2.8	3.1 ± 0.6^{abcde}	3.5 ± 0.6^{abc}	0.0 ± 0.0	3.3 ± 0.2^{abc}
PMC	3.4 ± 0.7^{abcd}	4.4 ± 1.7	3.6 ± 3.1	4.7 ± 2.5^{de}	2.0 ± 0.2^{ab}	0.0 ± 0.0	3.1 ± 1.7^{abc}
SA	4.3 ± 1.1^{cd}	4.4 ± 0.6	5.2 ± 1.9	2.5 ± 0.6^{abcde}	2.5 ± 1.7^{ab}	0.0 ± 0.0	3.0 ± 0.4^{abc}
SB	2.7 ± 0.3^{ab}	3.1 ± 2.4	4.3 ± 1.1	1.4 ± 0.5^{abc}	4.5 ± 1.7^{abcd}	0.0 ± 0.0	2.3 ± 0.3^{abc}
SC	4.2 ± 0.4^{bcd}	4.8 ± 2.4	6.0 ± 3.4	3.0 ± 1.4^{abcde}	4.0 ± 0.3^{abcd}	0.0 ± 0.0	3.1 ± 0.8^{abc}
SD	3.1 ± 0.6^{abcd}	5.8 ± 2.4	6.0 ± 1.2	3.0 ± 1.4^{abcde}	7.2 ± 3.2^{cde}	0.0 ± 0.0	2.0 ± 0.6^{abc}
UA	4.3 ± 1.2^{cd}	1.5 ± 0.5	4.4 ± 1.7	3.7 ± 0.9^{cde}	3.7 ± 1.5^{abc}	0.0 ± 0.0	6.0 ± 1.2^{d}
UB	4.2 ± 1.2^{bcd}	4.8 ± 3.5	3.1 ± 1.1	3.2 ± 3.0^{bcde}	5.0 ± 3.4^{bcd}	0.0 ± 0.0	4.7 ± 3.7^{cd}
UC	4.0 ± 1.4^{bcd}	3.9 ± 0.5	4.0 ± 2.2	2.4 ± 1.2^{abcde}	3.5 ± 2.8^{abc}	0.0 ± 0.0	2.9 ± 1.3^{abc}
UD	3.5 ± 3.0^{abcd}	6.9 ± 1.1	3.2 ± 2.4	2.1 ± 1.3^{abcd}	10.0 ± 4.6^{e}	0.0 ± 0.0	2.7 ± 0.3^{abc}
WA	2.3 ± 0.5^{a}	1.6 ± 0.2	2.5 ± 1.7	2.5 ± 1.7^{abcde}	0.50 ± 0.06^{a}	0.0 ± 0.0	4.8 ± 1.3^{cd}
WB	3.9 ± 1.7^{bcd}	4.5 ± 4.0	3.5 ± 4.0	3.4 ± 1.6^{bcde}	2.0 ± 0.2^{ab}	0.0 ± 0.0	2.6 ± 1.9^{abc}
WC	3.5 ± 0.6^{abcd}	6.7 ± 0.9	2.0 ± 0.2	0.97 ± 0.1^{ab}	8.0 ± 0.0^{de}	0.0 ± 0.0	1.6 ± 0.2^{a}
WD	2.8 ± 0.4^{abc}	4.4 ± 0.5	2.5 ± 0.3	0.50 ± 0.5^{a}	3.0 ± 0.3^{ab}	0.0 ± 0.0	2.1 ± 1.2^{abc}
P-value	0.023	0.266	0.394	0.017	<0.001	0.000	0.001

*Means with similar superscript down the group showed no significant differences (P>0.05)

Keys: CFU = Colony forming units; PM = Poultry Manure; S= Soil; U= Ugu (Pumpkin Leaf); W= Water Leaf; ABCD = Stations; THB = Total Heterotrophic Bacteria, TC = Total Coliform, TS = Total *Salmonella*, TSH = Total *Shigella*, TP = Total *Pseudomonas*, TV= Total *Vibrio*,TSC = Total *Staphylococcus*.

Citation: Odum *et al*. (2025). Bacteriological assessment of some vegetables and farm soils cultivated with poultry manure. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 191 – 200.

For distribution of bacterial isolates across pumpkin leaf, *Shigella* sp., *Pseudomonas* sp., *Bacillus* sp. and *Staphylococcus* sp. were distributed across the four sampling stations, *Salmonella sp*. was found in stations A, B, and C, *Enterobacter sp*. occurred in stations A, B, and D, while *Micrococcus sp*. occurred only in station D (Table 4a).

For percentage occurrence of bacterial isolates in pumpkin leaf, *Pseudomonas* sp., with 30% occurrence in station C had the highest percentage occurrence, followed by *Staphylococcus sp*., and *Bacillus* sp., with 26.1% occurrence in station D respectively, *Shigella sp*. with 23.8% occurrence in station B, *Salmonella sp*., with 23.1% occurrence in station A, *Salmonella sp*., and *Shigella* sp., with 20% occurrence in station C respectively, *Bacillus* sp., with 19.2% occurrence in station A, *Salmonella* sp., and *Pseudomonas* sp. each with 19% occurrence in station B respectively.

Table 4a: Distribution of Bacterial Isolates across Pumpkin Leaf Samples

Isolates	A	B	C	D
Salmonella spp.	+	+	+	-
Shigella spp.	+	+	+	+
Pseudomonas spp.	+	+	+	+
Bacillus spp.	+	+	+	+
Enterobacter spp.	+	+	-	+
Micrococcus spp.	-	-	-	+
Staphylococcus spp.	+	+	+	+

Keys: A, B, C = Manured Farm; D = Control; + = isolated; - = not isolated.

For the distribution of bacterial isolates across water leaf samples, *Shigella* spp, *Pseudomonas* spp, *Bacillus* spp, *Staphylococcus* spp, and *Klebsiella* spp, were distributed across the four sampling stations, *Salmonella* spp, was found in stations A, B, and C, *Enterobacter* spp, occurred in stations A, B, and D, while *Corynebacterium* spp, occurred in stations B and C (Table 4b).

For percentage occurrence of bacterial isolates in water leaf, *Bacillus* spp, and *Klebsiella* spp, with 25% occurrence in station D respectively, had the highest percentage occurrence, followed by *Shigella* spp, and *Bacillus* spp, each with 24.1% occurrence in station C.

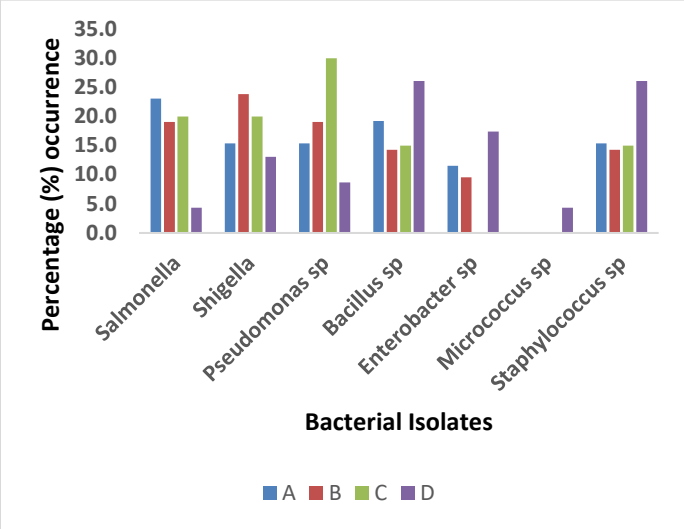

Fig. 1a: Percentage Occurrence of Bacterial Isolates in Pumpkin leaf Samples

Salmonella spp, with 22.2% occurrence in station A, *Shigella* spp, and *Staphylococcus* spp, with 18.8% occurrence in station D respectively. *Shigella* spp, and *Bacillus* spp, each with 18.5% occurrence in station A respectively. *Salmonella* spp., and *Klebsiella* spp, with 16.7% occurrence in station B respectively.

Table 4b: Distribution of Bacterial Isolates across Water Leaf Samples

Isolates	**A**	**B**	**C**	**D**
Salmonella spp.	+	+	+	-
Shigella spp.	+	+	+	+
Pseudomonas spp.	+	+	+	+
Bacillus spp.	+	+	+	+
Enterobacter spp.	+	+	-	+
Staphylococcus spp.	+	+	+	+
Corynebacterium spp.	-	+	+	-
Klebsiella spp.	+	+	+	+

Keys: A, B, C = Manured Farm; D = Control; + = isolated; - = not isolated.

For the distribution of bacterial isolates across soil samples (control), *Shigella* spp, *Pseudomonas* spp, *Bacillus* spp, *Staphylococcus* spp, and *Klebsiella* spp, were distributed across the four sampling stations, *Salmonella* spp, was found in stations A, B, and C, *Enterobacter* spp, occurred in stations A, B, and D, while *Corynebacterium* spp, occurred in stations B and C (Table 4c).

Citation: Odum *et al*. (2025). Bacteriological assessment of some vegetables and farm soils cultivated with poultry manure. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 191 – 200.

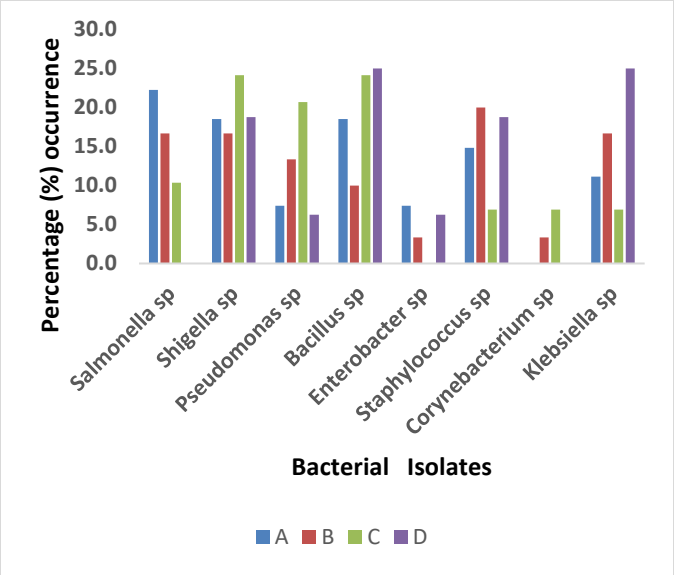

Fig. 1b: Percentage occurrence of bacterial isolates across water leaf samples

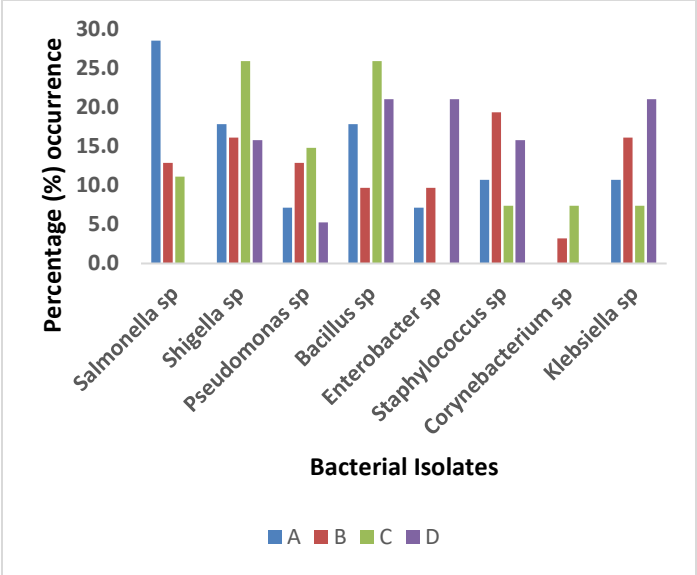

Fig. 1c: Percentage occurrence of bacterial isolates across soil samples

For percentage occurrence of bacterial isolates in soil sample/control (figure 1c), *Salmonella* spp, with 28.6% occurrence in station A had the highest percentage occurrence, followed by *Shigella* spp, and *Bacillus* spp, with 25.9% occurrence in station C, *Bacillus* spp, *Enterobacter* spp, and *Klebsiella* spp, with 21.1% occurrence in station D respectively, *Staphylococcus* spp, with 19.4% occurrence in station B, *Shigella* spp, and *Bacillus* spp, with 17.7% occurrence in station A respectively, *Shigella* spp, and *Klebsiella* spp, with 16.1% occurrence in station B respectively, among others.

Table 4c: Distribution of bacterial isolates across soil samples

Isolates	A	B	C	D
Salmonella spp.	+	+	+	-
Shigella spp.	+	+	+	+
Pseudomonas spp.	+	+	+	+
Bacillus spp.	+	+	+	+
Enterobacter spp.	+	+	-	+
Staphylococcus spp.	+	+	+	+
Corynebacterium spp.	-	+	+	-
Klebsiella spp.	+	+	+	+

Keys: A, B, C = Manured Farm; D = Control

The distribution of bacterial isolates across poultry manure samples are presented in Table 4.5d, *Salmonella* *Shigella* spp, *Pseudomonas* spp, *Bacillus* spp, and *Staphylococcus* spp, were distributed across the three sampling stations, *Micrococcus* spp, and *Enterobacter* spp, were found in stations A, and B, while *Corynebacterium* spp, occurred in stations B and C. For percentage occurrence of bacterial isolates in poultry manure sample (figure 4.2d), *Pseudomonas* spp, with 23.5% occurrence in station A had the highest percentage occurrence, followed by *Bacillus* spp, with 22.7% occurrence in station C, *Salmonella* spp, and *Staphylococcus* spp, with 19.4% occurrence in station B respectively, *Pseudomonas* spp, with 18.2% occurrence in station C, *Bacillus* spp, with 17.6% occurrence in station A, *Pseudomonas* spp, with 16.1% occurrence in station B, among others.

Table 4d: Distribution of bacterial isolates across poultry manure samples

Isolates	**A**	**B**	**C**
Salmonella spp.	+	+	+
Shigella spp.	+	+	+
Pseudomonas spp.	+	+	+
Micrococcus spp.	+	+	-
Bacillus spp.	+	+	+
Enterobacter spp.	+	+	-
Staphylococcus spp.	+	+	+
Corynebacterium spp.	-	+	+
Klebsiella spp.	+	+	+

Keys: A, B, C = Manured Farm.

Citation: Odum *et al*. (2025). Bacteriological assessment of some vegetables and farm soils cultivated with poultry manure. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 191 – 200.

Discussion

Fluted pumpkin leaves and other veggies are really vulnerable to contamination by microorganisms from soil, water, and handling during harvest or afterwards, and this means they can carry a variety of bacteria, including pathogens that affect both humans and plants (Osula *et al*., 2025). The results for bacterial load showed significant difference in the load of Total Heterotrophic Bacterial (THB) counts on pumpkin leaf, and water leaf in the different stations/farms when compared to the control indicating the effects of the application of poultry manure in the different farms. Similarly, Harry *et al*. (2023) in their study of antibiotic-resistant bacteria from organic fertilized farm soils and waterleaf in Aluu, Rivers State, Nigeria, reported a significant difference ($p<0.05$) in the total heterotrophic bacteria with values ranging from $2.72 \pm 0.52x10^6$ to $7.30 \pm 3.54x10^6$Cfu/g. Also, significant difference was recorded for Total Coliform counts (TCC), Total *Salmonella* counts (TSA), Total *Shigella* counts (TSH), and Total *Staphylococal* counts (TSC), in the farms where poultry manure was applied compared to the control, as well as on pumpkin leaf and water leaf for months 1 to month 3, indicating the effects of the application of poultry manure in the different farm. In the study by Osula *et al*., (2025), coliforms like *K. pneumoniae*, *E. coli*, *P. vulgaris*, and *E. cloacae* were reported in all the fluted pumpkin leaves and soil samples and *E. coli* was the most common coliform. This agrees with the studies by Igbinosa *et al*., (2023) and Lee *et al*., (2023) who reported that *E. coli* is often found in various vegetables. In line with findings in this study, Harry *et al*., (2023) reported a significant difference ($p < 0.05$) in *Staphylococcal,* and total coliform counts.

The bacterial load in this study matched those reported by Zarzecka *et al*., (2022). Also, they aligned with specific coliform counts from the studies of Ihechu *et al.,* (2023); Lee *et al*., 2023; Igbinosa *et al*., (2023). However, comparing these studies is tough because differences in laboratory methods, sampling, and handling can skew results (Martínez-Moreno *et al*., 2025). Still, using selective media with antibiotics or screening for bacteria has consistently shown a high presence of antibiotic-resistant bacteria on fresh produce (Osula *et al*., 2025). The presence of *Staphylococcus mascillensis*, *Corynebacterium sp*., *Shigella flexineri*, *Salmonella enterica*, *Bacillus cereus*, *Staphylococcus muscae*, *Micrococcus luteus*, *Escherichia coli*, *Staphylococcus gallinarum*, *Staphylococcus pettenkoferi*, *Enterobacter alburiae*, *Klebsiella pneumonae*, *Bacillus subtilis*, *Bacillus baduis*, *Bacillus smithii*, *Bacillus pantiothenicus*, *Staphylococcus lentus*, *Staphylococcus hyicus*, *Pseudomonas atronelloles*, *Bacillus samiensis*, *Staphylococcus jittensis*, and *Staphylococcus equorum* reported in this study have been reported in previous studies by Lennox and Nkra, (2016); Osula *et al*., (2025). The occurrence of *Bacillus* spp. in all the soils sampled and also as the most frequently occurring bacteria is an indication of the dominant habitation of soils by bacterial species specifically *Bacillus* (Yadav *et al*., 2015). This study has also shown that the soil can be a reservoir of gram-negative and gram-positive bacteria and agrees with the study of Taylor and Unakal, (2022). The occurrence of *Escherichia coli* having the highest occurrence could also be attributed to such factors as contamination between normal skin (hands, fingers, animal dropping, human dropping) flora, nasal discharge, soil, faecal matter used on the farm in form of manure as well as its ubiquitous distribution in the environment (Taylor and Unakal, 2022). Arguably, the prevalence of gram-negative and gram-positive bacteria in this study could be due to contaminated soil, poor storage, and farming practices (Liu *et al*., 2023). Also, the structure of fluted pumpkin leaves, with their folds and high surface area, might also attract these microorganisms (Igbinosa *et al*., 2023; Lee *et al*., 2023).

The most often reported harmful microorganisms linked to African fresh produce supply chains include *Staphylococcus aureus*, *Salmonella spp*., *Escherichia coli,* and *Listeria monocytogenes* (Somorin *et al*., 2023). This observation is not surprising and correlates with investigations from other regions (Ramirez-Hernandez *et al*., 2020; Townsend *et al*., 2021). The prevalence of pathogenic microorganisms, particularly those that are antimicrobial-resistant bacteria, in fruits and vegetables along the supply chain is, however, sparser than in other regions of the world (Somorin *et al*., 2023). In Africa today, fruit and vegetable consumption has increased significantly over the last 30 years (Mensah *et al*., 2021).

However, there is limited reliable data regarding the prevalence of pathogenic bacteria in fresh produce and epidemiological research linking these to outbreaks of foodborne illness throughout the continent (Imathui, 2018; Aworh, 2021).

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The consumption of food contaminated with microbes remains a significant health threat in the African region (WHO, 2015). Contributory factors include insufficient physical infrastructure (clean water, storage facilities, and transportation networks); inadequate awareness of food security issues and good manufacturing practices among relevant stakeholders (farmers, distributors, manufacturers, handlers, and consumers); and limited ability to develop and enforce food safety regulations (Jaffee *et al*., 2019).

Conclusion

In conclusion, although poultry manure improves soil fertility, applying it without treatment risks the microbiological safety of leafy vegetables. It is recommended that regulatory frameworks be strengthened, farmers be educated, and treated organic amendments be adopted to reduce pathogen transfer and safeguard consumers in Nigeria and similar agricultural environments.

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