

Physicochemical characteristics and faecal indicator organisms in composted animal wastes and waterleaf (*Talinum triangulare* (Jacq.)) cultivated in Calabar, Nigeria

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Abstract: The increasing use of animal wastes as organic soil amendments necessitates appropriate treatment to improve their physicochemical quality and reduce microbiological risks associated with agricultural application. This study evaluated the physicochemical characteristics of untreated, composted, and incinerated animal wastes and assessed faecal indicator organisms in manure-amended soils and *Talinum triangulare* (waterleaf) cultivated in Calabar, Nigeria. Fresh cow dung, poultry droppings, pig slurry, and their combinations were subjected to untreated, aerobic composting, and incineration treatments. Physicochemical parameters assessed included moisture content, organic matter, organic carbon, total nitrogen, pH, total solids, volatile solids, and dry matter. Faecal coliforms were enumerated in wastes, amended soils, and harvested waterleaf, while bacterial isolates were identified using standard microbiological methods. Selected representative *Escherichia coli* and *Salmonella* spp. isolates were characterised by PCR targeting the *uidA*, *invA*, and *hns* genes, respectively. Untreated wastes recorded higher moisture, organic matter, organic carbon, and nitrogen contents than treated wastes. Composting reduced moisture and faecal coliform loads while retaining more organic matter and nitrogen than incineration. Untreated manure-amended soils recorded the highest faecal coliform counts (5.30×10^5 – 2.10×10^6 CFU/g), compared with 1.50×10^3 – 4.23×10^3 CFU/g in composted treatments and 1.20×10^4 – 2.00×10^4 CFU/g in incinerated treatments. Similar reductions occurred in waterleaf cultivated with treated wastes. *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Proteus* spp., and *Salmonella* spp. were recovered, particularly from untreated treatments. PCR confirmed selected representative isolates, with expected *uidA* (147 bp), *invA* (284 bp), and *hns* (152–156 bp) products. Overall, composting substantially reduced faecal contamination while retaining more physicochemical properties than incineration, indicating its potential as a safer treatment option for agricultural use of animal wastes.

Keywords: Animal waste treatment, composting, incineration, physicochemical properties, *Talinum triangulare* (waterleaf).

INTRODUCTION

The increasing global demand for food, driven by rapid population growth, has intensified the use of synthetic fertilisers to enhance agricultural productivity. It is estimated that by 2050, the global population will reach approximately 9.1 billion, requiring about a 60% increase in food production to meet future food demands (Ortez, 2022). Although inorganic fertilisers have contributed significantly to increased crop yields, their prolonged use has been associated with soil degradation, nutrient

imbalance, environmental pollution, soil acidification, and declining long-term soil fertility (Karimi *et al.*, 2016; Bui and Nguyen, 2020). In addition, the high cost of chemical fertilisers limits their affordability for many smallholder farmers in developing countries, emphasising the need for sustainable and cost-effective alternatives (Abebe and Debebe, 2019; Ullah *et al.*, 2023).

Organic fertilisers derived from agricultural wastes have emerged as environmentally sustainable alternatives that

improve soil fertility while promoting circular waste management. Among the available waste treatment methods, composting is widely recognised as an effective approach for converting organic wastes into stable, nutrient-rich soil amendments suitable for agricultural use (Atienza-Martínez *et al.*, 2020; Waqas *et al.*, 2023). Mature compost improves soil structure, enhances water-holding capacity, increases nutrient availability, and supports beneficial microbial activity, thereby reducing dependence on synthetic fertilisers (Goldan *et al.*, 2023; Ullah *et al.*, 2023). Aerobic composting also substantially reduces pathogenic microorganisms through the heat generated during microbial decomposition, producing a safer organic amendment for agricultural application (Mehta and Sirari, 2018; FAO, 2021). Another management option is the incineration of animal wastes, which effectively eliminates pathogens but may reduce the nutrient content and agronomic value of the resulting material. Consequently, understanding the comparative effects of composting and incineration is important for selecting appropriate waste management strategies.

Talinum triangulare (waterleaf) is an important leafy vegetable widely cultivated and consumed in tropical Africa, particularly in Nigeria. Because it is commonly consumed fresh or minimally processed, the microbiological safety of waterleaf is an important public health concern. The application of inadequately treated organic manures may facilitate the transfer of faecal microorganisms from soil to edible plant tissues, increasing the risk of foodborne disease.

Despite their agronomic benefits, untreated animal wastes may contain enteric pathogens and faecal indicator organisms capable of contaminating soils and fresh vegetables, thereby posing risks to public health (Alegbeleye *et al.*, 2018; Chukwu *et al.*, 2022). The microbiological quality of organic amendments is therefore an important consideration before their application to agricultural soils. Faecal indicator organisms, particularly *Escherichia coli* and total coliforms, are commonly used to assess the hygienic quality of compost because their presence reflects faecal contamination and the effectiveness of waste treatment processes in reducing microbial hazards (Manyi-Loh *et al.*, 2016; Motlagh and Yang, 2019). Molecular confirmation of indicator organisms and selected pathogens further improves the accuracy of microbial identification and provides reliable evidence of the effectiveness of waste treatment methods in reducing potential health risks.

Animal wastes differ considerably in nutrient composition, carbon-to-nitrogen ratio, moisture content, and microbial populations, suggesting that different manure sources and treatment methods may vary in both fertiliser value and microbiological safety (Goldan *et al.*, 2023; Ullah *et al.*, 2023). Previous studies have examined the use of individual animal manures as organic fertilisers, the composting of livestock wastes, and the reduction of pathogenic microorganisms during manure treatment.

However, much of the available research has focused on individual waste streams or single treatment approaches, with limited comparative assessment of untreated, composted, and incinerated animal wastes. In particular, limited information is available on the comparative effects of these treatments on the physicochemical characteristics of cow dung, poultry droppings, pig slurry, and their combinations, alongside their microbiological quality following application to agricultural soil.

Furthermore, few studies have simultaneously assessed the occurrence of faecal indicator organisms in manure-amended soils and edible *Talinum triangulare* under humid tropical conditions in Nigeria. Addressing this gap is important because an amendment may retain desirable physicochemical properties while still posing microbiological risks to soil and fresh vegetables.

Therefore, this study evaluated the physicochemical characteristics of untreated, composted, and incinerated cow dung, poultry droppings, pig slurry, and their combinations, and assessed faecal indicator organisms in the corresponding manure-amended soils and *Talinum triangulare* (waterleaf) cultivated in Calabar, Nigeria. The study also used molecular techniques to confirm selected faecal indicator organisms and pathogenic bacteria. The findings provide comparative information on the physicochemical quality and microbiological safety of different animal-waste treatment methods and their suitability for use in vegetable production.

MATERIALS AND METHODS

Study area

The study was conducted in Calabar Metropolis, Cross River State, southern Nigeria (4.58°N, 8.21°E), at an elevation of approximately 62 m above sea level. Calabar is located within the humid tropical rainforest agro-ecological zone and experiences a long rainy season (March–November) and a short dry season (December–February). The area receives an average annual rainfall of approximately 3,000 mm, with mean annual temperatures ranging from 25 to 28°C (Amadi *et al.*, 2020). These climatic conditions are favourable for the year-round cultivation of leafy vegetables.

Experimental design and field layout

The field experiment was laid out in a randomised complete block design (RCBD) with four replicates. Treatments comprised untreated, composted, and incinerated animal wastes prepared from cow dung, poultry droppings, pig slurry, and their respective combinations. Each experimental plot measured 1 m × 1 m, with a 0.5 m buffer zone between plots to minimise nutrient movement and cross-contamination. Raised

edges were constructed around the plots to minimize runoff during rainfall and irrigation. The organic amendments were incorporated into the top 15–25 cm of soil before planting. Waterleaf (*Talinum triangulare*) was cultivated in all plots. Irrigation, manual weeding, and other agronomic practices were applied uniformly throughout the experimental period.

Collection and treatment of animal wastes

Fresh cow dung, poultry droppings, and pig slurry were collected from livestock farms within Calabar Metropolis, Cross River State, Nigeria, using sterile polyethylene bags and transported immediately to the experimental site. The collected wastes were allocated into three treatment groups: untreated, composted, and incinerated. The untreated wastes were applied directly to the designated experimental plots, while separate portions were subjected to aerobic composting and incineration to produce compost and ash, respectively, before field application.

Composting of animal wastes

Composting was carried out separately for cow dung, poultry droppings, pig slurry, and their respective combinations using the pit composting method. Compost pits were lined with wooden planks to prevent direct contact between the composting materials and the surrounding soil and covered with black polyethylene sheets to conserve heat and maintain suitable moisture conditions. Moisture content was maintained at approximately 50–60% by periodic addition of water, while the composting materials were manually turned once weekly to improve aeration and promote uniform decomposition. Composting was conducted for 30 days following the procedure described by Mengistu *et al.* (2017). The 30 days were selected based on the adopted composting procedure. At the end of the composting period, the physicochemical status of the compost was assessed based on moisture content, organic matter, organic carbon, total nitrogen, and pH before application to the experimental plots.

Incineration (burning) treatment

Animal wastes designated for incineration were first air-dried and subsequently combusted in an open-top metal burn barrel measuring approximately 0.6 m in diameter and 1.0 m in height. Combustion was conducted under open-air conditions until the material was converted to ash. No additional fuel was documented as being used during the combustion process. After cooling to room temperature, the ash was aseptically collected, transferred into sterile polyethylene bags, and stored under dry conditions until field application within 24 h (Chukwu *et al.*, 2022).

Determination of the physicochemical properties of organic manure

Representative compost samples were collected from the surface, centre, and bottom of each compost pit at 10-day intervals (days 10, 20, and 30) to monitor physicochemical changes during composting. The samples were placed in sterile plastic containers, transported immediately to the laboratory, and stored at 4°C until analysis. Physicochemical analyses were conducted at the Department of Chemistry Laboratory, while microbiological analyses were performed at the Department of Microbiology Laboratory, University of Cross River State, following standard analytical procedures.

Moisture content

Moisture content was determined according to the method described by Zobeashia *et al.* (2022). Briefly, 10 g of each compost sample was weighed into a pre-dried and pre-weighed crucible. The samples were dried at 98–100°C until a constant weight was obtained, cooled in a desiccator to room temperature, and reweighed. Moisture content was calculated from the loss in weight after drying using Equation (1).

$$\text{Moisture (\%)} = \frac{\Delta SB - \Delta SA}{\text{Weight of sample}} \times 100$$

Where: ΔSB = weight of dish and sample before drying, and ΔSA = weight of dish and sample after drying.

Total organic matter and organic carbon contents

Organic carbon was determined using a modified Springer and Klee method as described by Chukwu *et al.* (2021). Dried compost samples were finely ground and oxidised with 20 mL of 2 N potassium dichromate ($K_2Cr_2O_7$) and 26 mL of concentrated sulfuric acid (H_2SO_4) at $160 \pm 2^\circ C$. The excess dichromate was titrated with ferrous sulfate ($FeSO_4$) solution, and the percentage organic carbon (P_{OC}) was calculated using Equation (2):

$$POC = \frac{(A - B) \times F \times K}{W}$$

Where; A is the volume of $FeSO_4$ solution (mL) used in titrating excess $K_2Cr_2O_7$ of the sample, B is the volume of $FeSO_4$ solution (mL) used in titrating excess $K_2Cr_2O_7$ of the blank analysis, F is the correction factor from daily titration of $K_2Cr_2O_7$ solution with $FeSO_4$ (1.30), K is a constant given by the dilution and equivalent C content (0.003×100), and W is the weight of the sample (g). The value of P_{OM} was then calculated from P_{OC} as

$$P_{OM} = 0.35 + 1.80 \times P_{OC}$$

Total nitrogen

Total nitrogen (TN) was determined using the Kjeldahl digestion method as described by El Asri *et al.* (2020). Briefly, dried compost samples were ground to pass through a 2-mm sieve and digested with concentrated sulfuric acid (H₂SO₄) in the presence of a catalyst mixture. The digested samples were subjected to steam distillation, and the liberated ammonia was collected and quantified by titration. The total nitrogen content was expressed as a percentage of dry weight (Mengistu *et al.*, 2017; El Asri *et al.*, 2020).

Total solid

The total solid content of the substrate was determined by drying a known volume of the substrate in a pre-weighed crucible dish at 105°C in a hot air oven for one hour. After which, it was left to cool at room temperature in a desiccator and weighed (Zobeashia *et al.*, 2022). The TS was computed using the following formula:

$$TS = \frac{M_1 - M_2}{V}$$

Where: M₁: mass of crucible dish after drying at 105 °C (mg), M₂: mass of initial crucible dish (mg) V: Volume of sample (L)

Volatile solid

Volatile solids are the amount of solid that volatilizes when heated at 550 °C. It was estimated by burning the total solid at 550°C for about 2 hours in a muffle furnace. The crucible was taken out and allowed to cool in a desiccator and then weighed (Zobeashia *et al.*, 2022). The VS was determined using the formula:

$$VS = \frac{M_1 - M_3}{V}$$

Where: M₁: mass of crucible dish after drying at 105 °C (mg), M₃: Mass of crucible dish after ignition at 550 °C (mg), V: Volume of sample (L)

Dry matter

Twenty (20 g) of organic waste was dried at 110 °C to constant mass (24 hours). The percentage dry matter content was measured from the weight of the sample before and after drying:

$$P_{dm} = \frac{(W_{dw} + W_c)}{((W_{fw} + W_c) - W_c)} \times 100$$

Where: W_{dw} , W_c and W_{fw} are the weights of the dried sample, container and undried sample respectively (all in g).

Determination of temperature and pH

The pH of animal wastes was determined according to the AOAC (2012) method. Air-dried samples were sieved, and 30 g was mixed with 30 ml distilled water to form a slurry. The mixture was allowed to stand for 1 h with intermittent stirring.

Isolation and Identification of Faecal Indicator Organisms

Faecal indicator organisms were isolated from untreated, composted, and incinerated animal wastes, manure-amended soils, and *Talinum triangulare* (waterleaf) using standard microbiological procedures. Soil samples were collected three days after manure application from a depth of 10 cm. Two soil samples were collected from each experimental plot using a sterile soil auger, transferred into sterile polyethylene bags, and transported immediately to the laboratory for analysis. Similarly, two mature waterleaf leaves were aseptically harvested from each plot, placed in sterile polyethylene bags without prior washing, and transported to the laboratory for microbiological analysis.

Under aseptic conditions, 10 g of each manure or soil sample, or 10 g of chopped waterleaf leaves, was homogenised in 90 mL of sterile distilled water to obtain an initial suspension. The homogenates were thoroughly mixed, and ten-fold serial dilutions were prepared up to 10⁻⁶. Aliquots (0.1 mL) of appropriate dilutions were spread, in triplicate, onto Eosin Methylene Blue (EMB) agar, MacConkey agar, and Salmonella–Shigella (SS) agar. The inoculated plates were incubated at 37°C for 24–48 h.

Following incubation, colonies exhibiting characteristic morphology on the selective media were purified by repeated streaking onto nutrient agar to obtain pure cultures. Purified isolates were maintained on nutrient agar slants at 4°C until further identification.

Biochemical identification of faecal indicator organisms

Pure bacterial isolates were characterised based on colonial morphology, Gram staining, and a series of biochemical tests following standard microbiological procedures. The biochemical tests included oxidase, catalase, indole, methyl red (MR), Voges–Proskauer (VP), citrate utilisation, urease, and triple sugar iron (TSI) tests.

Isolates presumptively identified as *Escherichia coli* were Gram-negative rods that were indole-positive, citrate-negative, and urease-negative. Presumptive *Salmonella* spp. isolates were Gram-negative rods that were indole-

negative, citrate-positive, and urease-negative. Representative isolates with these biochemical characteristics were selected for molecular confirmation.

Enumeration of faecal coliforms

Faecal coliform counts were determined according to the method described by Mengistu *et al.* (2017). Briefly, 10 g of each untreated, composted, and incinerated manure sample was homogenised in 90 mL of sterile distilled water under aseptic conditions. The suspension was thoroughly mixed, and serial ten-fold dilutions were prepared up to 10^{-6} .

An aliquot (0.1 mL) from appropriate dilutions was spread, in triplicate, onto Eosin Methylene Blue (EMB) agar and incubated at 44°C for 24 h. After incubation, characteristic faecal coliform colonies were counted, and results were expressed as $\log_{10}$ colony-forming units per gram ($\log_{10}$ CFU/g) of sample.

Molecular characterisation of selected faecal indicator organisms

DNA extraction

Representative isolates presumptively identified as *Escherichia coli* and *Salmonella* spp. by cultural, morphological, and biochemical methods were selected for molecular confirmation. Genomic DNA was extracted from overnight bacterial cultures using the boiling method. Briefly, bacterial cells were harvested by centrifugation, suspended in sterile nuclease-free water, heated at 95°C for 10 min, and immediately chilled on ice. Cell debris was removed by centrifugation, and the resulting supernatant containing genomic DNA was used as the PCR template.

PCR amplification

Species-specific PCR assays were performed to confirm selected faecal indicator organisms. Eight representative *Escherichia coli* isolates recovered from untreated animal wastes and waterleaf samples were screened for the *uidA* gene. Three representative *Salmonella* spp. isolates obtained from untreated poultry droppings and waterleaf samples were screened for the *invA* and *hns* genes.

PCR amplification was carried out in a final reaction volume of 25 μ L containing 12.5 μ L of 2 $\times$ PCR Master Mix, 1.0 μ L each of forward and reverse primers (10 pmol μ L⁻¹), 2.0 μ L of template DNA, and nuclease-free water to volume. Amplification was performed in a thermal cycler using an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, primer annealing at the appropriate primer-specific temperature, extension at 72°C for 45 s, and a final extension at 72°C for 5 min. Annealing temperatures of 58–60°C, 64°C, and 58°C were used for the *uidA*, *invA*, and *hns* genes,

respectively (Bej *et al.*, 1991; Frahm and Obst, 2003; Rahn *et al.*, 1992). Positive and negative controls were included in each PCR run.

Agarose gel electrophoresis

PCR products were resolved by electrophoresis on 1.5% agarose gels prepared in 1 $\times$ Tris-acetate-EDTA (TAE) buffer and stained with an appropriate nucleic acid stain. Electrophoresis was carried out at 100 V for approximately 45 min alongside a 100 bp DNA ladder. DNA bands were visualised using a gel documentation system, and amplicon sizes were determined by comparison with the molecular weight marker. The expected PCR product sizes were approximately 147 bp for the *uidA* gene, 284 bp for the *invA* gene, and 156 bp for the *hns* gene.

Cultivation and sampling of waterleaf

Organic amendments comprising untreated animal wastes, composts, and incinerated manure ash were incorporated into the top 15–25 cm of soil before planting. Waterleaf (*Talinum triangulare*) seeds obtained from the Cross River State Agricultural Input Supply Agency were sown at a depth of approximately 1.3 cm. All plots received uniform irrigation, manual weeding, and general agronomic management throughout the study.

For microbiological analysis, two mature leaves were aseptically harvested from each plot at the designated sampling period, transferred into sterile polyethylene bags without washing, and transported immediately to the laboratory for analysis.

Statistical analysis

Data were analysed using IBM SPSS Statistics version 20. Microbial counts were transformed to $\log_{10}$ values before analysis. Descriptive statistics were used to summarise the physicochemical characteristics and faecal indicator organism counts across the different animal-waste treatments. Microbial counts are presented as CFU/g, and observed differences among treatments were interpreted descriptively.

RESULT

Physicochemical characteristics of untreated, composted, and incinerated animal wastes

Moisture Content

Moisture content varied considerably among animal waste types and treatment methods (Table 1). Untreated wastes recorded the highest moisture contents, with pig slurry exhibiting the greatest values (80–85%), followed by cow dung (78–82%) and poultry droppings (70–75%). Mixed

Table 1. Moisture content (%) of animal wastes under different treatment methods.

Treatment	Untreated (%)	Composted (%)	Incinerated (%)
Cow dung	78–82	45–50	5–8
Poultry droppings	70–75	40–45	4–7
Pig slurry	80–85	50–55	6–10
Cow dung + Pig slurry	79–83	47–52	5–8
Pig slurry + Poultry droppings	78–82	45–50	5–8
Poultry droppings + Cow dung	77–81	44–49	5–7
Cow dung + Poultry droppings + Pig slurry	78–82	46–51	6–9

Table 2. Organic matter and organic carbon content (%) of animal wastes under different treatment methods.

Treatment	Untreated OM (%)	Untreated OC (%)	Composted OM (%)	Composted OC (%)	Incinerated OM (%)	Incinerated OC (%)
Cow dung	88–90	51–53	65–70	38–40	15–18	9–10
Poultry droppings	85–88	50–52	60–65	35–37	12–15	7–9
Pig slurry	87–90	51–54	62–67	36–39	13–16	7–10
Cow dung + Pig slurry	88	51	65	38	15	9
Pig slurry + Poultry droppings	87	50	63	37	14	8
Poultry droppings + Cow dung	86	49	61	36	12	7
Cow dung + Poultry droppings + Pig slurry	87	50	64	38	13	8

Table 3. Total nitrogen content (%) of animal wastes under different treatment methods.

Treatment	Untreated (%)	Composted (%)	Incinerated (%)
Cow dung	2.5–2.8	1.8–2.1	0.2–0.4
Poultry droppings	3.0–3.3	2.0–2.3	0.3–0.5
Pig slurry	2.7–3.0	1.9–2.2	0.2–0.4
Cow dung + Pig slurry	2.6–2.9	1.9–2.2	0.2–0.4
Pig slurry + Poultry droppings	2.8–3.1	2.0–2.3	0.3–0.5
Poultry droppings + Cow dung	2.7–3.0	1.9–2.2	0.2–0.4
Cow dung + Poultry droppings + Pig slurry	2.8–3.1	2.0–2.3	0.3–0.5

untreated wastes showed similar moisture contents (77–83%). Composting reduced moisture content across all treatments to 40–55%, whereas incineration resulted in the lowest moisture contents (4–10%).

Organic matter and Organic Carbon Content

Organic matter (OM) and organic carbon (OC) contents varied according to waste type and treatment method (Table 2). Untreated wastes recorded the highest OM and OC values, with pig slurry and cow dung showing the highest organic content (87–90% OM and 51–54% OC). Composting resulted in moderate reductions, with composted materials retaining 60–70% OM and 35–40% OC. Incineration reduced OM to 12–18% and OC to 7–10%. Mixed waste treatments followed a similar pattern,

with composted combinations maintaining higher organic content than incinerated wastes.

Total nitrogen content

Total nitrogen (TN) content varied among animal wastes and treatment methods (Table 3). Untreated wastes recorded the highest nitrogen levels, with poultry droppings showing the greatest content (3.0–3.3%), followed by pig slurry (2.7–3.0%) and cow dung (2.5–2.8%). Mixed waste treatments showed comparable nitrogen values, with the combined cow dung, poultry droppings, and pig slurry treatment recording among the highest levels (2.8–3.1%). Composting resulted in moderate nitrogen reductions, with treated wastes retaining 1.8–2.3% nitrogen. In contrast, incineration reduced nitrogen levels to 0.2–0.5%.

Table 4. Total Solids (TS), Volatile Solids (VS), and Dry Matter (DM) of Animal Wastes under Different Treatment Methods.

Treatment	Untreated TS/DM (%)	Composted TS/DM (%)	Incinerated TS/DM (%)	Untreated VS (%)	Composted VS (%)	Incinerated VS (%)
Cow dung	17–21	48–52	91–95	15–18	10–12	3–5
Poultry droppings	18–22	45–50	90–94	16–19	11–13	2–4
Pig slurry	18–22	46–50	92–95	15–18	10–12	3–5
Cow dung + Pig slurry	17–22	47–52	91–94	15–17	10–11	3–4
Pig slurry + Poultry droppings	18–23	45–50	90–94	16–18	10–12	2–4
Poultry droppings + Cow dung	17–21	46–50	91–94	15–18	10–12	2–4
Cow dung + Poultry droppings + Pig slurry	18–23	46–51	91–95	16–18	10–12	3–4

Table 5. pH of animal wastes under different treatment methods.

Treatment	Untreated pH	Composted pH	Incinerated pH
Cow dung	6.5–6.8	7.2–7.8	8.0–8.2
Poultry droppings	6.2–6.7	7.0–7.6	8.0–8.3
Pig slurry	6.3–6.8	7.2–7.7	8.1–8.4
Cow dung + Pig slurry	6.4–6.8	7.2–7.8	8.0–8.2
Pig slurry + Poultry droppings	6.3–6.7	7.1–7.7	8.1–8.3
Poultry droppings + Cow dung	6.3–6.8	7.1–7.6	8.0–8.2
Cow dung + Poultry droppings + Pig slurry	6.3–6.8	7.2–7.8	8.1–8.4

Total solids, volatile solids and dry matter

Total solids (TS), volatile solids (VS), and dry matter (DM) varied according to treatment method (Table 4). Untreated animal wastes recorded the lowest TS and DM values (17–23%), reflecting their high moisture contents. Composting increased TS and DM to 45–52%, whereas incineration produced the highest values (90–95%). In contrast, volatile solids progressively declined with treatment. Untreated wastes recorded the highest VS values (15–19%), followed by composted wastes (10–13%), while incinerated wastes exhibited the lowest VS values (2–5%), indicating substantial loss of organic matter during thermal treatment.

pH

The pH of animal wastes varied significantly with treatment method (Table 5). Untreated wastes exhibited slightly acidic to neutral pH values (6.2–6.8). Composting increased pH values to neutral or slightly alkaline conditions (7.0–7.8). Incineration produced strongly alkaline pH values (8.0–8.4). Similar patterns were observed in mixed waste treatments, where composting enhanced stabilisation through increased pH, while incineration caused greater alkalinity due to mineral concentration after combustion.

Faecal indicator organisms in soils amended with untreated, composted and incinerated animal wastes

Faecal coliform load in soils amended with untreated, composted and incinerated animal wastes

The faecal coliform counts in soils amended with untreated, composted, and incinerated animal wastes are presented in Table 6. Soils amended with untreated animal wastes recorded the highest faecal coliform loads, ranging from $5.30 \pm 0.32 \times 10^5$ CFU/g in cow dung-amended soils to $2.10 \pm 0.15 \times 10^6$ CFU/g in soils amended with the combined cow dung, poultry droppings, and pig slurry treatment. Among the untreated manures, poultry droppings, pig slurry, and mixed manure treatments generally exhibited higher faecal coliform counts than cow dung alone.

Composting markedly reduced faecal coliform loads across all manure types, with mean counts ranging from $1.50 \pm 0.30 \times 10^3$ to $4.23 \pm 0.31 \times 10^3$ CFU/g. The lowest count was recorded in composted cow dung, whereas the highest occurred in the composted mixture of cow dung, poultry droppings, and pig slurry.

The lowest faecal coliform counts were observed in soils amended with incinerated animal wastes, where values ranged from $1.20 \pm 0.40 \times 10^1$ to $2.00 \pm 0.50 \times 10^1$ CFU/g. Both composting and incineration substantially reduced faecal coliform loads compared with untreated animal

Table 6. Faecal coliform counts in soils amended with untreated, composted and incinerated animal wastes

Treatment	Faecal coliform count (CFU/g, Mean $\pm$ SD)
Untreated animal wastes	
Cow dung	$5.30 \pm 0.32 \times 10^5$
Poultry droppings	$1.30 \pm 0.10 \times 10^6$
Pig slurry	$1.63 \pm 0.15 \times 10^6$
Cow dung + Pig slurry	$1.77 \pm 0.15 \times 10^6$
Pig slurry + Poultry droppings	$1.97 \pm 0.15 \times 10^6$
Poultry droppings + Cow dung	$1.63 \pm 0.15 \times 10^6$
Cow dung + Poultry droppings + Pig slurry	$2.10 \pm 0.15 \times 10^6$
Composted animal wastes	
Cow dung	$1.50 \pm 0.30 \times 10^3$
Poultry droppings	$2.30 \pm 0.30 \times 10^3$
Pig slurry	$3.33 \pm 0.31 \times 10^3$
Cow dung + Pig slurry	$3.73 \pm 0.31 \times 10^3$
Pig slurry + Poultry droppings	$4.10 \pm 0.30 \times 10^3$
Poultry droppings + Cow dung	$3.50 \pm 0.30 \times 10^3$
Cow dung + Poultry droppings + Pig slurry	$4.23 \pm 0.31 \times 10^3$
Incinerated animal wastes	
Cow dung	$1.50 \pm 0.50 \times 10^1$
Poultry droppings	$1.20 \pm 0.40 \times 10^1$
Pig slurry	$1.87 \pm 0.61 \times 10^1$
Cow dung + Pig slurry	$1.80 \pm 0.40 \times 10^1$
Pig slurry + Poultry droppings	$2.00 \pm 0.40 \times 10^1$
Poultry droppings + Cow dung	$1.60 \pm 0.40 \times 10^1$
Cow dung + Poultry droppings + Pig slurry	$2.00 \pm 0.50 \times 10^1$

Note: Values are presented as mean $\pm$ standard deviation (SD) of four replicates (n = 4).

Table 7. Percentage occurrence of faecal indicator organisms recovered from soils amended with different animal waste treatments.

Treatment	Predominant faecal indicator organisms (% occurrence)
Untreated cow dung	<i>Escherichia coli</i> (33.3), <i>Enterobacter</i> spp. (33.3), <i>Klebsiella</i> spp. (33.3)
Untreated poultry droppings	<i>Klebsiella</i> spp. (45.5), <i>Escherichia coli</i> (27.3), <i>Enterobacter</i> spp. (27.3)
Untreated pig slurry	<i>Klebsiella</i> spp. (41.7), <i>Enterobacter</i> spp. (33.3), <i>Escherichia coli</i> (25.0)
Untreated cow dung + pig slurry	<i>Klebsiella</i> spp. (46.7), <i>Escherichia coli</i> (26.7), <i>Enterobacter</i> spp. (26.7)
Untreated pig slurry + poultry droppings	<i>Klebsiella</i> spp. (41.7), <i>Enterobacter</i> spp. (33.3), <i>Escherichia coli</i> (25.0)
Untreated cow dung + poultry droppings	<i>Escherichia coli</i> (40), <i>Klebsiella</i> spp. (40), <i>Enterobacter</i> spp. (20)
Untreated cow dung + poultry droppings + pig slurry	<i>Escherichia coli</i> (25.9), <i>Klebsiella</i> spp. (16.7), <i>Enterobacter</i> spp. (16.7), <i>Proteus</i> spp. (16.7)
Composted cow dung	<i>Escherichia coli</i> (33.3), <i>Enterobacter</i> spp. (33.3), <i>Citrobacter</i> spp. (33.3)
Composted poultry droppings	<i>Escherichia coli</i> (37.5), <i>Klebsiella</i> spp. (25.0), <i>Enterobacter</i> spp. (37.5)
Composted pig slurry	<i>Escherichia coli</i> (50.0%), <i>Enterobacter</i> spp. (50.0%)
Other composted treatments	<i>Escherichia coli</i> , <i>Enterobacter</i> spp., <i>Klebsiella</i> spp., <i>Citrobacter</i> spp. (25.0–66.7%)
Incinerated treatments	<i>Enterobacter</i> spp., <i>Klebsiella</i> spp. (50.0–100%)

wastes, with incineration producing the greatest reduction.

Occurrence of faecal indicator organisms in soils amended with different animal waste treatments

The distribution of faecal indicator organisms recovered from soils amended with the different animal waste

treatments is presented in Table 7. The organisms identified included *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp., while *Salmonella* spp. was recovered only from soils amended with selected untreated mixed animal wastes.

Soils amended with untreated animal wastes exhibited the highest diversity and frequency of faecal indicator organisms. *Escherichia coli* predominated in soils

Table 8. Faecal coliform counts in *Talinum triangulare* cultivated in soils amended with untreated, composted and incinerated animal wastes.

Treatment	Mean Faecal Coliform Count (CFU/g)
Untreated animal wastes	
Cow dung	$2.55 \pm 0.12 \times 10^3$
Poultry droppings	$4.88 \pm 0.24 \times 10^3$
Pig slurry	$6.12 \pm 0.24 \times 10^3$
Cow dung + Pig slurry	$7.32 \pm 0.32 \times 10^3$
Pig slurry + Poultry droppings	$8.00 \pm 0.40 \times 10^3$
Cow dung + Poultry droppings	$7.08 \pm 0.39 \times 10^3$
Cow dung + Poultry droppings + Pig slurry	$8.77 \pm 0.46 \times 10^3$
Composted animal wastes	
Cow dung	$1.50 \pm 0.30 \times 10^3$
Poultry droppings	$1.83 \pm 0.30 \times 10^3$
Pig slurry	$1.20 \pm 0.20 \times 10^3$
Cow dung + Poultry droppings	$1.95 \pm 0.29 \times 10^3$
Cow dung + Pig slurry	$1.30 \pm 0.20 \times 10^3$
Poultry droppings + Pig slurry	$1.20 \pm 0.10 \times 10^3$
Cow dung + Poultry droppings + Pig slurry	$1.10 \pm 0.10 \times 10^3$
Incinerated animal wastes	
Cow dung	5×10^2
Poultry droppings	4×10^2
Pig slurry	5×10^2
Cow dung + Poultry droppings	6×10^2
Cow dung + Pig slurry	7×10^2
Poultry droppings + Pig slurry	5×10^2
Cow dung + Poultry droppings + Pig slurry	$6.5 \pm 0.50 \times 10^2$

Values are expressed as mean $\pm$ standard deviation (SD) of replicate samples where applicable.

amended with untreated cow dung (33.3%) and the combined cow dung, poultry droppings, and pig slurry treatment (25.9%), whereas *Klebsiella* spp. showed higher frequencies in soils amended with untreated poultry droppings (45.5%) and pig-based waste mixtures (41.7–46.7%). The high prevalence of these organisms reflects the substantial microbial burden associated with the application of untreated animal manures.

The frequency and diversity of faecal indicator organisms were markedly reduced in soils amended with composted animal wastes. *Escherichia coli* remained the predominant organism in soils receiving composted cow dung (33.3%), composted poultry droppings (37.5%), and mixed compost (40.0%). However, *Enterobacter* spp., *Citrobacter* spp., and *Klebsiella* spp. were also isolated, indicating that some enteric bacteria were capable of surviving the composting process and persisting in amended soils, albeit at lower frequencies than in untreated manure-amended soils.

Minimal recovery of faecal indicator organisms was observed in soils amended with incinerated animal wastes.

Faecal Indicator organisms recovered from *Talinum triangulare* cultivated in soils amended with untreated, composted and incinerated animal wastes

Faecal coliform counts in Talinum triangulare cultivated in soils amended with untreated, composted and incinerated animal wastes

Faecal coliforms were recovered from *Talinum triangulare* cultivated in soils amended with untreated, composted, and incinerated animal wastes (Table 8). Plants cultivated in soils amended with untreated animal wastes recorded the highest faecal coliform counts, ranging from $2.55 \pm 0.12 \times 10^3$ to $8.77 \pm 0.46 \times 10^3$ CFU/g. The lowest count among the untreated treatments was recorded in plants grown in soil amended with untreated cow dung ($2.55 \pm 0.12 \times 10^3$ CFU/g), whereas the highest count was observed in plants grown in soil amended with the combined cow dung, poultry droppings, and pig slurry treatment ($8.77 \pm 0.46 \times 10^3$ CFU/g).

Faecal coliform counts were lower in plants cultivated in

Table 9. Faecal Coliform counts and predominant faecal indicator organisms recovered from *Talinum triangulare* cultivated with untreated and treated animal wastes.

Manure Treatment	Predominant organisms detected	Percentage occurrence (%)
Untreated cow dung	<i>E. coli</i> , <i>Enterobacter</i> sp., <i>Klebsiella</i> sp.	50, 25, 25
Untreated poultry droppings	<i>E. coli</i> , <i>Salmonella</i> sp., <i>Klebsiella</i> sp.	40, 40, 20
Untreated pig slurry	<i>E. coli</i> , <i>Enterobacter</i> sp., <i>Proteus</i> sp.	40, 40, 20
Untreated cow dung + pig slurry	<i>E. coli</i> , <i>Enterobacter</i> sp., <i>Proteus</i> sp., <i>Citrobacter</i> sp.	40, 20, 20, 20
Untreated pig slurry + poultry droppings	<i>Salmonella</i> sp., <i>E. coli</i> , <i>Klebsiella</i> sp., <i>Proteus</i> sp.	40, 20, 20, 20
Untreated cow dung + poultry droppings	<i>E. coli</i> , <i>Klebsiella</i> sp., <i>Enterobacter</i> sp.	40, 40, 20
Untreated cow dung + poultry droppings + pig slurry	<i>E. coli</i> , <i>Klebsiella</i> sp., <i>Enterobacter</i> sp., <i>Proteus</i> sp.	50, 16.7, 16.7, 16.7
Composted cow dung	<i>E. coli</i> , <i>Enterobacter</i> sp.	66.7, 33.3
Composted poultry droppings	<i>E. coli</i> , <i>Enterobacter</i> sp., <i>Klebsiella</i> sp.	50, 25, 25
Composted pig slurry	<i>E. coli</i> , <i>Enterobacter</i> sp.	50, 50
Composted mixed manure treatments	<i>E. coli</i> , <i>Enterobacter</i> sp., <i>Klebsiella</i> sp., <i>Citrobacter</i> sp.	25–66.7
Incinerated manure treatments	<i>Enterobacter</i> sp., <i>Klebsiella</i> sp.	50–100

soils amended with composted animal wastes, ranging from $1.10 \pm 0.10 \times 10^3$ to $1.95 \pm 0.29 \times 10^3$ CFU/g. The lowest counts were observed in vegetables cultivated in soils amended with incinerated animal wastes, ranging from 4×10^2 to 7×10^2 CFU/g.

Faecal indicator organisms in *Talinum triangulare* cultivated with untreated and treated animal wastes

The occurrence of faecal indicator organisms recovered from *Talinum triangulare* cultivated in soils amended with untreated, composted, and incinerated animal wastes is presented in Table 9. The organisms identified included *Escherichia coli*, *Salmonella* spp., *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., and *Citrobacter* spp.

Vegetables cultivated in soils amended with untreated animal wastes showed the highest diversity of faecal indicator organisms. *Escherichia coli* was the predominant organism in most untreated manure treatments, accounting for 40–50% of the isolates, while *Salmonella* spp. occurred mainly in vegetables cultivated in soils amended with untreated poultry droppings and pig slurry–poultry droppings mixtures. *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., and *Citrobacter* spp. were also recovered from untreated manure treatments.

The occurrence and diversity of faecal indicator organisms decreased in vegetables cultivated in soils amended with composted animal wastes. *Escherichia coli* remained the predominant organism, whereas *Enterobacter* spp., *Klebsiella* spp., and *Citrobacter* spp. were recovered at lower frequencies.

Vegetables cultivated in soils amended with incinerated animal wastes yielded the fewest bacterial isolates. Only *Enterobacter* spp. and *Klebsiella* spp. were recovered,

indicating that thermal treatment substantially reduced the occurrence of faecal indicator organisms associated with animal waste application.

Molecular characterisation of selected faecal indicator organisms

PCR amplification of the *uidA* gene in *Escherichia coli*

PCR targeting the *uidA* gene (β -glucuronidase), a species-specific molecular marker for *Escherichia coli*, was performed on eight representative *E. coli* isolates recovered from untreated animal wastes and waterleaf samples. Amplification was carried out using the *uidA*-specific forward primer F (5'-AAAACGGCAAGAAAAAGC AG-3') and reverse primer R (5'-ACGCGTGGTTACAGTC TTGCG-3'), which target an expected amplicon of approximately 147 bp. Gel electrophoresis of the PCR products revealed that all eight isolates produced clear, distinct ~147 bp amplicons, corresponding to the band obtained with the positive control. No amplification was observed in the negative control, confirming the specificity of the assay and the molecular confirmation of the *E. coli* isolates (Table 10, Figure 1).

PCR amplification of the *invA* Gene in *Salmonella* spp.

PCR targeting the *invA* gene (invasion gene A), a conserved virulence-associated gene of *Salmonella* spp., was performed on three representative *Salmonella* spp. isolates obtained from untreated poultry droppings and waterleaf samples. Amplification was carried out using the *invA*-specific forward primer F (5'-GTGAAATTATCGCCA

Table 10. PCR amplification of the *uidA* gene in representative *Escherichia coli* Isolates.

Source of Isolate	No. Tested	<i>uidA</i> -Positive	Negative Control	Positive Control
Untreated cow dung	2	2	0	1
Untreated poultry droppings	2	2	0	1
Untreated pig slurry	2	2	0	1
<i>Talinum triangulare</i> grown in untreated manure-amended soil	2	2	0	1

Table 11. PCR amplification of the *invA* Gene in representative *Salmonella* spp. Isolates.

Source of Isolate	No. Tested	<i>invA</i> -Positive	Negative Control	Positive Control
Untreated poultry droppings	2	2	0	1
<i>Talinum triangulare</i> grown in untreated manure-amended soil	1	1	0	1

Table 12. PCR Amplification of the *hns* Gene in Representative *Salmonella* spp. Isolates.

Source of Isolate	No. tested	<i>hns</i> -Positive	Negative Control	Positive Control
Untreated poultry droppings	2	2	0	1
<i>Talinum triangulare</i> grown in untreated manure-amended soil	1	1	0	1

CGTTCGGGCAA-3') and reverse primer R (5'-TCATCGCACCGTCAAAGGAACC-3'), which target an expected amplicon of approximately 284 bp. Gel electrophoresis of the PCR products showed that all three isolates produced distinct bands corresponding to the expected *invA* amplicon. The positive control produced the expected amplification, while no amplification was observed in the negative control, confirming the specificity and reliability of the PCR assay for molecular confirmation of *Salmonella* spp. (Table 11, Figure 1).

PCR amplification of the *hns* Gene in *Salmonella* spp.

PCR targeting the *hns* gene (histone-like nucleoid structuring gene), a conserved gene used for molecular confirmation of *Salmonella* spp., was performed on the same *Salmonella* spp. isolates as described above. Amplification was carried out using the *hns*-specific forward primer F (5'-TACCAAAGCTAAACGCGCAGCT-3') and reverse primer R (5'-TGATCAGGAAATCTTCCAGTTGC-3'), targeting an expected amplicon of approximately 156 bp. Gel electrophoresis of the PCR products revealed distinct amplicons corresponding to the expected *hns* product in the three isolates. The positive control produced the expected amplification, while no amplification was observed in the negative control, confirming the specificity and reliability of the PCR assay for molecular confirmation of *Salmonella* spp. (Table 12, Figure 1).

DISCUSSION

The findings of this study show that treatment methods influenced the physicochemical characteristics and microbiological quality of animal wastes and the occurrence of faecal indicator organisms in amended soils and *Talinum triangulare*. Untreated animal wastes recorded high moisture contents, with pig slurry showing the highest values, followed by cow dung and poultry droppings. The high moisture content observed in untreated wastes is consistent with the characteristics of freshly generated livestock manure, which typically contains considerable water from faecal and urinary components. High moisture content may limit oxygen penetration and create conditions that favour the persistence of anaerobic and enteric microorganisms within manure matrices. Similar observations have been reported by Jahan *et al.* (2022), who noted that livestock wastes generally possess high moisture content that influences decomposition dynamics and microbial survival.

Composting resulted in a considerable reduction in moisture content across all manure types, whereas incineration produced the lowest moisture values. The decline following composting may be associated with microbial decomposition and water loss during the composting process. Reduced moisture content is an important characteristic of stabilised compost because it improves handling and storage properties. Roy *et al.* (2024) similarly reported that moisture reduction during composting occurs as microorganisms degrade organic

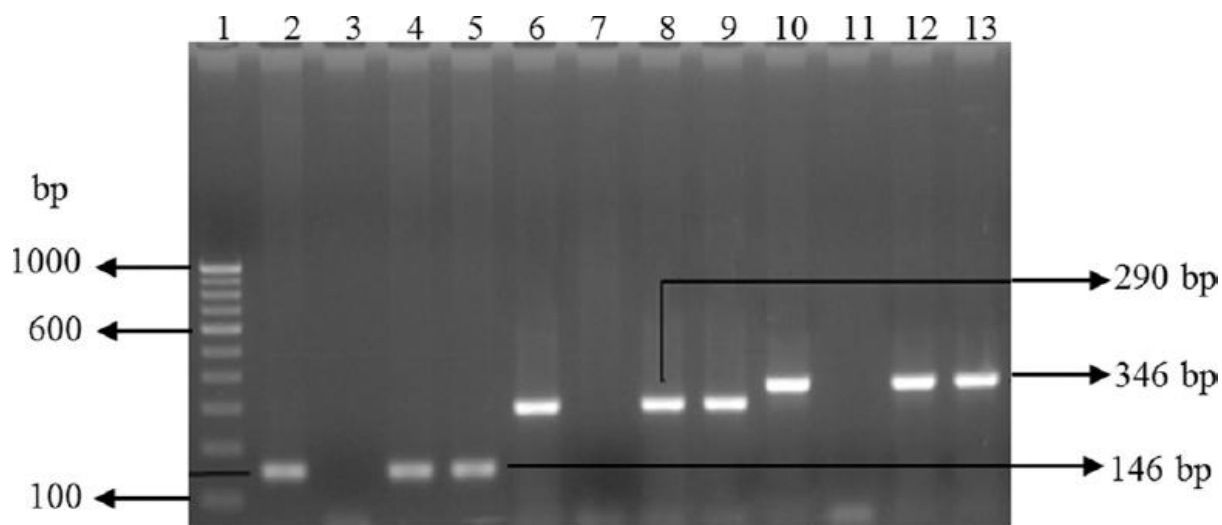


Figure 1. Gel electrophoresis showing PCR amplification of target genes in *Escherichia coli* and *Salmonella* spp. isolates from untreated animal wastes and waterleaf. Lane 1: 100 bp DNA ladder; lane 2: *uidA* gene-positive control (*E. coli*); lane 3: *uidA* gene-negative control (*E. coli*); lanes 4 and 5: representative *uidA*-positive *E. coli* isolates; lane 6: positive control for the *invA* gene (*Salmonella*); lane 7: negative control for *invA*; lanes 8 and 9: representative *invA*-positive *Salmonella* spp. isolates; lane 10: positive control for the *hns* gene (*Salmonella*); lane 11: negative control for the *hns* gene; lanes 12 and 13: representative *hns*-positive *Salmonella* spp. isolates.

substrates and promote water loss. The extremely low moisture values observed after incineration reflect extensive dehydration and conversion of organic materials into dry ash following thermal treatment.

The organic matter and organic carbon contents of untreated wastes were higher than those of composted and incinerated treatments. The reduction in organic matter following composting may be associated with microbial mineralisation of carbonaceous compounds, resulting in carbon dioxide release and stabilisation of the remaining organic fractions. Similar reductions in organic carbon during compost maturation have been reported by Noor *et al.* (2024). Although composting reduces readily decomposable organic fractions, it can increase the stability of the remaining organic material. In contrast, the substantial depletion of organic matter following incineration is attributable to combustion of organic compounds, leaving predominantly inorganic mineral residues. This reduction may limit the ability of incinerated manure products to contribute organic matter to amended soils compared with composted materials.

Nitrogen content varied among the manure treatments, with untreated poultry droppings recording the highest nitrogen concentration. This finding agrees with previous reports that poultry manure contains relatively high nitrogen levels because of the presence of nitrogen-rich compounds in poultry excreta. Composting resulted in some reduction in nitrogen content, which may be associated with ammonia volatilisation, microbial immobilisation, and other nitrogen transformations occurring during decomposition. Liu *et al.* (2015) reported similar nitrogen losses during composting of organic

wastes. Incineration resulted in the greatest reduction in nitrogen content, which may be associated with volatilisation and thermal decomposition of nitrogen-containing compounds. This observation is consistent with Shan *et al.* (2021), who reported substantial nutrient losses during combustion of organic wastes.

Changes in total solids, volatile solids, and dry matter content further reflected the effects of the treatment processes. Composting increased total solids and dry matter content, which may be attributed largely to moisture loss during decomposition, whereas incineration produced the highest values because of extensive dehydration and concentration of mineral residues. The reduction in volatile solids following treatment indicates the degradation or removal of biodegradable organic fractions. These findings are consistent with previous studies identifying volatile-solids reduction as an indicator of organic matter decomposition and stabilisation during waste treatment (Jahan *et al.*, 2022).

Microbiological evaluation revealed marked differences in faecal coliform loads among the manure treatments. Untreated wastes recorded the highest faecal coliform counts, with pig slurry and poultry-based manure mixtures generally recording higher loads than cow dung. The higher counts observed in mixed manure treatments may be related to the combined microbial contribution of the different animal waste sources. Similarly, high levels of faecal indicator organisms in untreated livestock manure have been reported in agricultural environments, emphasising the microbiological risks associated with direct application of raw manure to crop-production soils (Chukwu *et al.*, 2022).

Composting was associated with a marked reduction in faecal coliform populations compared with corresponding untreated manure treatments. Based on the values presented in Table 6, composting produced approximately 99.72–99.82% reductions, corresponding to about 2.55–2.75 $\log_{10}$ reductions in faecal coliform counts across the manure treatments. Incineration produced even greater reductions of approximately 99.997–99.999%, corresponding to about 4.55–5.03 $\log_{10}$ reductions compared with the corresponding untreated treatments. The reduction associated with composting may be related to microbial competition, depletion of readily available nutrients, prolonged treatment, and other unfavourable conditions during compost maturation. Composting has been widely recognised as an effective approach for reducing enteric bacteria, including *Escherichia coli* and other faecal indicators (Manga *et al.*, 2020). However, the recovery of low levels of faecal indicator organisms from some composted treatments indicates that microbial elimination may depend on the duration and management of the composting process. Incineration recorded the lowest faecal coliform counts, consistent with the effectiveness of thermal treatment in reducing bacterial populations. However, the associated loss of organic matter and nitrogen may reduce the value of the resulting ash as an organic soil amendment.

The application of treated and untreated animal wastes influenced the microbiological quality of amended soils. Soils receiving untreated manure exhibited the highest faecal coliform loads and greater diversity of bacterial isolates, including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Proteus* spp., and *Salmonella* spp. The predominance of *E. coli* is consistent with its abundance in the gastrointestinal tract of livestock and its established use as an indicator of faecal contamination. The detection of *Salmonella* spp. in soils amended with untreated poultry-containing manure is of particular public-health relevance because of the potential for transfer to crops. Previous studies have similarly reported the persistence and movement of enteric microorganisms following application of untreated manure to agricultural soils (Bui and Nguyen, 2020).

The occurrence of faecal indicator organisms in *Talinum triangulare* also varied according to manure treatment. Vegetables cultivated with untreated manure recorded higher faecal coliform counts and greater diversity of bacterial isolates than those receiving composted and incinerated treatments. The recovery of *E. coli*, *Salmonella* spp., *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., and *Citrobacter* spp. from vegetables grown with untreated manure indicates the possibility of contamination through manure-amended soil and other environmental pathways. Leafy vegetables may be particularly susceptible because of their large surface area and proximity to soil particles. Similar observations have been reported for leafy vegetables cultivated using untreated organic amendments (Chukwu *et al.*, 2022).

Composted manure treatments were associated with lower faecal coliform counts and reduced bacterial diversity in *Talinum triangulare* compared with untreated treatments. Although organisms such as *E. coli*, *Enterobacter* spp., *Klebsiella* spp., and *Citrobacter* spp. were occasionally recovered, their lower occurrence compared with untreated treatments is consistent with reduced microbial contamination following composting. Vegetables cultivated with incinerated manure recorded the lowest bacterial loads, with limited recovery of *Enterobacter* spp. and *Klebsiella* spp. The recovery of these organisms after incinerated-manure treatment may reflect post-treatment environmental contamination; however, the present study did not directly distinguish between environmental contamination and survival following treatment.

Molecular analysis further supported the identity of the selected representative isolates recovered during the study. All eight selected representative *E. coli* isolates produced the expected *uidA* gene amplification, while the three selected representative *Salmonella* spp. isolates produced the expected *invA* and *hns* gene amplification. The *uidA* gene, which encodes β -glucuronidase, is widely used as a molecular marker for *E. coli* detection (Frahm and Obst, 2003). Similarly, *invA* is a conserved gene widely used as a molecular target for *Salmonella* detection (Rahn *et al.*, 1992). The agreement between the biochemical characterisation and PCR results supports the identification of the selected representative isolates.

Overall, the findings indicate that untreated animal wastes carry a greater microbiological burden when applied directly to agricultural soils, whereas composting and incineration reduce faecal indicator organisms to different extents. Incineration produced the greatest reduction in faecal coliform counts but also resulted in substantial losses of organic matter and nitrogen. Composting provided a substantial reduction in faecal contamination while retaining more of the organic and nutrient characteristics of the manure. Therefore, composting appears to offer a more balanced waste-treatment option where both microbiological safety and retention of organic amendment properties are important for agricultural application.

Conclusion

This study showed that treatment methods influenced the physicochemical characteristics and microbiological quality of animal wastes used for agricultural application. Untreated wastes recorded high faecal coliform loads and a greater occurrence of faecal indicator organisms, indicating potential microbiological risks associated with their direct application to agricultural soils. Composting reduced faecal contamination while retaining more organic matter and nitrogen than incineration. Incineration produced the greatest reduction in culturable faecal

coliforms but resulted in substantial losses of organic components. Molecular analysis further confirmed the identity of selected representative *Escherichia coli* and *Salmonella* spp. isolates using the *uidA*, *invA*, and *hns* genes, respectively. Under the conditions of this study, composting represents a potentially suitable treatment option for reducing microbiological risks while retaining more of the physicochemical properties of animal wastes than incineration.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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