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Impact of animal dung on soil pH and microbiota: A study of two medium-scale livestock farms in Ibadan, Oyo state, Nigeria

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Abstract

This study investigated the impact of animal-dung on soil pH and microbiota. Soil samples from five different livestock litters were collected from two medium-scale livestock farms in Ibadan, Oyo-State. These included litter-free soil with pH 7 (control), and soil samples from dungs of poultry, pig, goat and cattle. Physicochemical properties of each soil sample were assessed according to Association Official Analytical Chemist (AOAC) procedures. Total bacterial count and total fungi count of each sample were determined using standard pour plate technique. Fungi and soil health-indicator bacteria including cellulolytic, amylolytic, proteolytic, phosphate-solubilizing, nitrogen-fixing bacteria and actinobacteria were isolated and identified using cultural characteristics, biochemical tests and molecular techniques. Data obtained were subjected to descriptive statistics and analysis of variance (ANOVA) at $p = 0.05$. Results showed that pH value was lowest (5.48) in cattle dung soil and highest (5.78) in pig dung soil. Cellulolytic, amylolytic, proteolytic, phosphate-solubilizing, nitrogen-fixing bacteria and actinobacteria were identified. Microbial analyses showed that soil samples from pig dung had relatively higher count (65.5×10^6 CFU/ml) of heterotrophic bacteria and total fungi count (15.75×10^6 CFU/g) than other soil samples. Isolates subjected to molecular identification were identified as *P. aeruginosa*, *B. cereus*, *K. pneumoniae*, *B. subtilis* and *B. thuringiensis* with 99% similarity search identity. Statistical analysis revealed that relationship exists between the physicochemical parameters and microbes isolated as $r > 0$. This study established soil acidification caused by poorly managed litters which impact soil microbiota.

Keywords: Livestock farms, Soil acidification, Soil health, Soil microbiota, Oyo State, Nigeria

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INTRODUCTION

Livestock holds substantial importance in the livelihoods of rural communities and economies of developing nations. They serve as income and employment for farmers. They offer a crucial source of animal protein, contributing significantly to the nutritional needs of billions of households, both rural and urban. In urban and peri-urban regions, the primary types of livestock farms include cattle, pigs, broiler and layer chickens, as well as various other poultry farms, along with sheep and goat farms (Wong *et al.*, 2017). The litters associated with each of these livestock have been used either as the primary fertilizer or in combination with traditional inorganic fertilizers.

The scarcity of land in the urban and peri-urban regions has led to the need for intensifying animal production systems, which involves confining animals to smaller spaces. As the number of livestock rises, there has been a corresponding increase in the generation of animal wastes (Dopelt *et al.*, 2019). In urban livestock units with limited space, the substantial production of animal wastes can lead to significant environmental as well as public health issues if not properly disposed or managed (Singh and Rashid, 2017). Consequently, this accelerated growth negatively impacts the environment.

It has been established that continuous animal waste disposal on a land contributes to the acidification of the soil, and soil pH influences nutrient availability. In highly acidic soils, leaching leads to the depletion of potassium, calcium, and magnesium (Fischer and Dubis, 2019). Additionally, a deficiency of calcium can cause structural problems in the soil. This rise in soil acidity leads to a reduction in cation exchange capacity, mobilization of aluminum ions, and a decrease in biological activity (FAO, 2007). This can alter the distribution, diversity, and abundance of soil microorganisms (flora and fauna) which in turn affects the soil structure and functionality (Martínez-Mera *et al.*, 2017). Improper handling of litter waste can also result in the accumulation of nitrogen and phosphorus on the soil surface, followed by their transport into ground and surface water. This can lead to a deterioration in water quality and an elevation in soil acidity (Xu *et al.*, 2018). Acidic precipitation in soils with livestock wastes that are not properly managed exert detrimental effects on soil microorganisms (Xu, *et al.*, 2018). An essential component of agricultural ecosystems are soil organisms. These organisms may vary from large animals and plants to tiny microbes (Sekaran *et al.*, 2021). Because soil-dwelling organisms are

responsible for biogeochemical transformations, biological diversity is essential for supporting soil functionality (Pino *et al.*, 2019). The preservation of fertile soils and productive fields for agriculture depends on the presence of a diversified variety of soil organisms. Soil microbes play a crucial role in providing numerous ecosystem services including: litter degradation, nutrient mineralization, nutrient cycling, breakdown of pollutants and pesticides, nitrogen fixation, carbon sequestration, fostering a healthy soil structure for plant growth, facilitating rainwater infiltration, detoxifying contaminants, and regulating pathogenic agents. (Somenahally *et al.*, 2021; Singh *et al.*, 2021)

Many research studies have been carried out on the effect of waste disposal over years on soil pH and microbial population and diversity (van der Bom *et al.*, 2018; Zhen *et al.*, 2014). But there has not been any study on impact of animal dungs on the selected sites of study. Therefore, this study aimed to assess the impact of livestock dungs on soil acidity and soil microbiota: a study of two medium-scale livestock farms in Ibadan, Oyo state

MATERIALS AND METHODS

Experimental design and sampling

The research design was a completely randomized design. Soils exposed to five different treatments: T1- litter free soil with pH between 6-7; T2- top soil with poultry litter; T3- top soil with pig excreta, T4: top soil with goat excreta, T5: top soil with cattle dung, were used for the study with each treatment replicated thrice. The soil samples were collected from two medium scale livestock farms in Ibadan north and Ido local government area of Ibadan, Oyo state, Nigeria. These locations are the teaching and research farms of the University of Ibadan and Moor plantation farms, Apata, Ibadan. Soil samples from the top 0–10 cm were collected and homogenized following standard procedure (AOAC, 2005). Approximately 500 g of soil sample from each site was kept in individual Ziploc bag, labelled and instantly stored in an ice pack. For long-term storage, samples were frozen in a -20°C freezer upon arrival at the laboratory.

Physicochemical analyses of soil samples

The following parameters which are pointers to healthiness of any soil were assessed following standard procedures: pH, Ca^{2+} , Mg^{2+} , Na^{+} , K , Mo^{6+} , Al^{3+} , Cu^{2+} , Mn^{2+} , % organic C, % Total N and % organic matter (AOAC, 2005). The

organic carbon and organic matter were determined by the wet oxidation method of Mingorance *et al.* (2007). The crude protein content in the samples were determined using the routine semi-micro Kjeldahl procedure (AOAC, 2005), which involves three analytical techniques: digestion, distillation, and titration.

Microbial analyses of soil samples

Total bacterial count (TBC) and total fungi count (TFC) of each sample were determined using standard pour plate technique as described by Habib *et al.* (2021). One (1) ml of dilution 10^6 of each sample was used for each TBC and TFC

Isolation of bacteria from soil samples

Isolation of the bacteria which help to maintain soil health was carried out on different media following standard procedures. These bacteria included cellulolytic, nitrogen-fixing, phosphate-solubilizing, actinomycetes, amylolytic, and proteolytic bacteria. The isolation method employed by Habib *et al.* (2021) was adopted for isolating cellulolytic bacteria. One ml of dilution 10^{-6} was used to inoculate a Bushnell Haas medium (BHM) and incubated at 37 °C for 48hrs. Another one ml of dilution 10^{-6} was used to inoculate Ashby nitrogen-free agar plate and incubated at 28 °C for 48 hours to isolate nitrogen-fixing bacteria as described by Zhang *et al.* (2022). Pikovskayas Agar M520 was used to isolate phosphate solubilizing bacteria as described by Panhwar, *et al.* (2014). Starch Casein Agar employed by Sowmya and Ramalingappa (2022) was adopted to isolate actinomycetes from the soil samples. Amylolytic bacteria were isolated from a starch-containing medium as described by Arvinder *et al.* (2012) while proteolytic bacteria were isolated on a medium containing skim milk as described in the procedure of Olutiola *et al.* (1991).

Isolation and identification of fungi from soil samples

One gram of each soil samples was weighed under sterile condition and mixed with 9ml of sterile distilled water. This was used to prepare serial dilutions from 10^{-1} to 10^{-6} . One (1) ml of dilution 10^{-6} was inoculated into sterile petri dish on which molten potato dextrose agar (PDA) was added. After which was allowed to solidify and incubated at $28 \pm 2^\circ\text{C}$ for 72 - 96 hours until visible growth was observed from the plates. The fungal colonies were subcultured until pure culture was obtained. The pure cultures of various isolated fungi were identified using

structural features such as colony, colour, extent of growth, presence or absence of mycelia, spores and nature of colony surface.

Characterization of isolates from soil samples

Bacterial isolates from the soil samples were further characterized using different biochemical tests which included indole, Citrate hydrolysis, catalase, urease, oxidase, hydrogen sulfide production, gelatin liquefaction and starch hydrolysis. Five bacterial isolates were selected for molecular characterization due to paucity of fund.

Molecular identification of isolates using 16S rRNA gene sequencing

The DNA from each isolate were extracted using Cetyl trimethyl ammonium bromide (CTAB) procedure as described by Turaki *et al.* (2017). PCR analysis, which was run with the universal primer for bacteria and purification of PCR sequencing products were carried out following the method described by Olowe *et al.* (2017). The sequencing was carried out and a blast was made on the NCBI gene bank.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) at $p=0.05$. Means were separated using Duncan multiple range test of the SPSS analytical software. A correlation analysis was carried out to investigate the degree of association among physicochemical variables in soil samples and also to determine the relationship between the physicochemical parameters and microbial composition of the soil.

RESULTS

Table 1 shows the sample analyses of soils as affected by different livestock excreta. Calcium level in T4 (8.31 cmol/kg) was significantly ($p<0.05$) higher compared to other treatments. Magnesium in T4 was 6.40 cmol/kg and was significantly ($p<0.05$) higher than other treatments. However, the lowest Mg value was observed in T1 (1.42). Sodium concentration was highest in T5 (0.97) and T1 had the lowest value of 0.61. Potassium value in T5 was 1.95cmol/kg and was the highest. Percentage total nitrogen (PTN) was lowest ($p<0.05$) in the control (0.25) and highest in T4 (0.74). Percentage organic carbon (POC) was significantly ($p<0.05$) higher in T4 (1.61) and T3

(1.58) compared to other treatments. Percentage organic M was highest in T4 (2.77) and lowest in T1 (1.91). Available phosphorus (mg/kg) was lowest in T1 (11.54) and highest in T4 (17.06). The Mn in T4 (11.06) was significantly ($p < 0.05$) higher compared to other treatments. Aluminum value was higher in soils on T4 (0.40 mg/kg) and value gradually decreased to 0.31 in T2; to 0.25 (T3), to 0.11 (T1). Copper was highest in T4 (0.73) and lowest in T1 (0.37). The Mo value observed in T4 was 0.45mg/kg and it was significantly ($p < 0.05$) higher compared to other treatments. The pH of soil samples as affected by different livestock excreta is shown in Figure 1. It was observed that the control pH was 7.00 and differed significantly from the pH values recorded for poultry (5.67), pig (5.78), cattle (5.48), and goat (5.70). Table 2 shows the

microbial population of soils affected by different livestock excreta. The T1 had the highest ($p < 0.05$) total bacterial count value of 82.00×10^6 CFU/ml compared to T2 (41.00), T3 (65.50), T4 (24.50) and T5 (51.00). Higher ($p < 0.05$) total fungi count was observed in T1 (18.50×10^6 CFU/g) compared to other treatments. However, T5 (6.50) had the lowest TFC. The cellulolytic bacteria were highest in T1 (40.50×10^6 CFU/ml) and lowest in T5 (14.50×10^6 CFU/ml). The phosphate solubilizing bacteria in T2 (69.50×10^6 CFU/ml) were significantly ($p < 0.05$) higher compared to other treatments. The nitrogen-fixing bacteria were highest ($p < 0.05$) in T3 (12.50×10^6 CFU/ml) and lowest in T1 (4.90) and T5 (4.75). Actinobacteria count in T3 was 35.50×10^6 CFU/ml and was significantly ($p < 0.05$) higher than other treatments.

Table 1: Physiochemical Analyses of soil samples from different livestock dung

Parameters	Control (T1)	Poultry(T2)	Pig (T3)	Cattle (T4)	Goat (T5)	SEM	P value
Ca (cmol/kg)	3.04 ^e	6.21 ^d	6.88 ^c	8.31 ^a	6.96 ^b	0.59	0.000
Mg (cmol/kg)	1.42 ^d	4.21 ^c	4.61 ^b	6.40 ^a	4.68 ^b	0.54	0.000
Na (cmol/kg)	0.61 ^d	0.81 ^c	0.91 ^b	0.94 ^{ab}	0.97 ^a	0.44	0.000
K (cmol/kg)	1.36 ^d	1.76 ^c	1.81 ^c	1.88 ^b	1.95 ^a	0.69	0.000
% total N	0.25 ^d	0.58 ^b	0.46 ^c	0.74 ^a	0.54 ^{bc}	0.54	0.001
% org C	1.11 ^d	1.44 ^c	1.58 ^{ab}	1.61 ^a	1.53 ^b	0.06	0.000
% org M	1.91 ^d	2.48 ^c	2.72 ^{ab}	2.77 ^a	2.64 ^b	0.10	0.000
Av. P (mg/kg)	11.54 ^e	15.46 ^d	16.67 ^b	17.06 ^a	16.56 ^c	0.68	0.000
Mn (mg/kg)	7.63 ^e	9.64 ^d	9.91 ^b	11.06 ^a	9.77 ^c	0.37	0.000
Al (mg/kg)	0.11 ^d	0.31 ^b	0.25 ^c	0.40 ^a	0.22 ^c	0.33	0.000
Cu (mg/kg)	0.37 ^d	0.60 ^c	0.66 ^b	0.73 ^a	0.59 ^c	0.04	0.000
Mo (mg/kg)	0.16 ^d	0.38 ^b	0.32 ^c	0.45 ^a	0.38 ^b	0.03	0.000

^{abcde}Means of treatments along a row with different superscripts differed significantly ($p < 0.05$). Ca-calcium, Mg-magnesium, Na-sodium, K-potassium, N-nitrogen, Org C-organic carbon, Av. P- Available phosphorus, Mn-manganese, Al-aluminum, Cu-copper, Mo-Molybdenum.

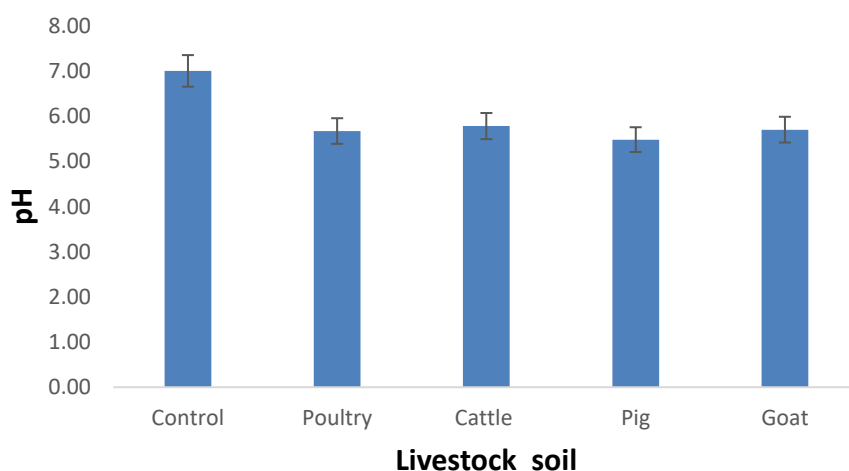


Figure 1: pH of soil samples from different livestock dung

Table 2: Microbial population of different soils as affected by different livestock excreta

Parameters (x 10 ⁶ cfu/ml)	Control (T1)	Poultry (T2)	Pig (T3)	Cattle (T4)	Goat (T5)	SEM	P value
TBC	82.00 ^a	41.00 ^d	65.50 ^b	24.50 ^e	51.00 ^c	6.60	0.000
TFC (cfu/g)	18.50 ^a	11.50 ^c	15.75 ^b	11.50 ^c	6.50 ^d	1.38	0.000
CBC	40.50 ^a	18.50 ^d	36.00 ^b	25.50 ^c	14.50 ^e	3.32	0.000
PSBC	23.50 ^c	69.50 ^a	25.50 ^b	18.75 ^d	24.85 ^{bc}	6.23	0.000
NFBC	4.90 ^d	8.90 ^b	12.50 ^a	7.75 ^c	4.75 ^d	0.96	0.001
Actinobacteria	5.50 ^e	20.50 ^b	35.50 ^a	15.50 ^e	9.50 ^d	3.48	0.000

^{abcde} Means of treatments along a row with different superscripts differed significantly (p<0.05)

KEY: TBC- Total bacterial count; TFC- Total fungi count; CBC- Cellulolytic bacteria count; PSBC- Phosphate solubilizing bacteria count; NFBC- Nitrogen fixing bacteria count

Table 3: Characteristics and properties of partial 16s ribosomal rna sequences of selected bacteria isolated from litter soils at the University of Ibadan, Oyo State, Nigeria

Sample organism	Strain	Accession No	No of nucleotides	Highest nBLAST identity (%)	E-value	Alignment score	Highest query coverage (%)
<i>P. aeruginosa</i>	UI-ANSCB-01	OP320372	1,408	99.65	0.00	≥200	100
<i>B. cereus</i>	UI-ANSCB-02	OP320373	1,438	99.93	0.00	≥200	100
<i>K.pneumonia</i>	UI-ANSNFB-03	OP320374	1,485	99.73	0.00	≥200	100
<i>B. subtilis</i>	UI-ANSPSB-04	OP320375	1,402	99.71	0.00	≥200	100
<i>B.thuringiensis</i>	UI-ANSAC-05	OP320376	1,444	99.86	0.00	≥200	100

Table 3 shows the characterization and properties of partial 16S ribosomal RNA sequences of selected five bacteria derived from litter soils at the University of Ibadan, Oyo State, Nigeria using nBLAST on GenBank. It was observed that *Pseudomonas aeruginosa* strain (UI-ANSCB-01) share the closest homology with sequence with accession numbers OP320372 at 99.65%, with 1408 number of nucleotides. *Bacillus cereus* strain (UI-ANSCB-02) share the closest homology with sequence with accession number OP320373 at 99.93%. *Klebsiella pneumoniae* strain (UI-ANSNFB-03) share the closest homology with sequence with accession number OP320374 at 99.73% with 1485 number of nucleotides. *Bacillus subtilis* strain (UI-ANSPSB-04) share the closest homology with sequence with accession number OP320375 at 99.71% identity. *Bacillus*

thuringiensis strain (UI-ANSAC-05) share the closest homology with sequence with accession number OP320376 at 99.86 % identity. Presented in Table 4 are correlation coefficients showing relationship between physicochemical properties and microbial load. There exists a high degree of relationship between pH, cellulolytic bacteria count (0.866) and actinobacteria count (0.866); while zero correlation exist between pH and TBC, TFC, PSBC, NFBC. Relationship between Mg to all microbial counts except CBC and actinobacteria, is strong and negative. Perfect and significant (p<0.01) relationship exists between K, Mn, Cu, Mo and all microbial counts except CBC which is relatively weak (0.5). Aluminum is negatively correlated (though not significant) with all microbe count, but positive and strong with CBC and actinobacteria

Table 4: Relationship between physicochemical parameters and microbial analyses of each soil sample from livestock dungs

	TBC	TFC	CBC	PSBC	NFBC	ACTINO BC
pH	0	0	0.866	0	0	0.866
Ca (cmol/kg)	0.961	0.961	0.721	0.961	0.961	-0.24
Mg (cmol/kg)	-0.721	-0.721	0.24	-0.721	-0.721	0.961
Na (cmol/kg)	-0.721	-0.721	-0.961	-0.721	-0.721	-0.24
K (cmol/kg)	1.000**	1.000**	0.5	1.000**	1.000**	-0.5
Mn (mg/kg)	1.000**	1.000**	0.5	1.000**	1.000**	-0.5
Al (mg/kg)	-0.327	-0.327	0.655	-0.327	-0.327	0.982
Cu (mg/kg)	-1.000**	-1.000**	-0.5	-1.000**	-1.000**	0.5
Mo (mg/kg)	1.000**	1.000**	0.5	1.000**	1.000**	-0.5

DISCUSSION

In this study, the impact of livestock dungs on soil acidity and soil microbiota was investigated. The result on Table 1 shows that the T1 value for Ca, Mg, N, C, P, Mn, Al, Cu and Mo differ significantly from other treatments. Soil pH influences nutrient availability. In strongly acidic soils, potassium, calcium and magnesium are depleted due to leaching which is evident when the values for T2, T3, T4 and T5 were compared. Acids attack soil minerals and increase net loss of nutrients such as Mn, Cu, Zn from the soil. This result aligns with a previous study that highlighted the potential occurrence of metal toxicity in acidic conditions (Clarholm *et al.*, 2015). In such circumstances, the active microbial population and their involvement in the processes of N, P, and S immobilization/mineralization may be hindered. Additionally, acidic conditions can lead to calcium deficiency, significantly impacting symbiotic N₂ fixation. Moreover, acidic soils with low organic matter content tend to have poor tilth (Jackson and Meetei, 2018). However, in comparing the litter soils with control (litter free soil), one would expect that these elements will be in higher quantity in litter free soil compared to the littered soils, however, the reverse is the case. This could be because the litter free soil is nutrient deficient because litters are not dumped on it.

The major causes for soils to become acidic are organic matter decomposition, rainfall and leaching, acidic parent material, nitrification of ammonium, and low buffer capacity from little clay and organic matter (Fischer and Dubis, 2019).

A soil is considered acidic when it is rich in acidic cations, like hydrogen (H⁺) and aluminium (Al³⁺) compared to the alkaline cations, such as calcium (Ca²⁺), magnesium (Mg²⁺), potassium (K⁺) and sodium (Na⁺) (Tahat *et al.*, 2020).

The result on Figure 1 shows that the pH value for the T1 differs significantly from the values recorded for poultry, pig, cattle and goat. One of the factors that contribute to the acidification of the soil is improper disposal of livestock litters that has taken place over time. When hydrogen ions replace basic elements held by soil colloids like calcium, magnesium, sodium, and potassium, the soils become acidic. In some cases, these waste's litter and manure components are used as an organic fertilizer and has a high nutritional value, recycling nutrients like nitrogen, phosphorus, and potassium. Litters and manure especially from poultry due to the high concentrations of protein and amino acids, contains significant concentrations of organic nitrogen; about 60–80% of the nitrogen in fresh manure, and is usually in the form of organic compounds like urea and protein (Lichuan, 2022).

Also, the presence of ammonium ions contributes to the acidic pH (Liu *et al.*, 2021). The results of present study were consistent with a study which reported that a decrease in soil pH occurs when acid-forming nitrogen fertilizers are used continuously (Parihar *et al.*, 2019). Another study mentioned that increase in the acidity of the soils resulted from organic fertilizer application, and majority of the territory had a pH below 4 (Homyak, *et al.*, 2018).

Due to the essential role of soil microorganisms in nutrient cycling, higher microbial biomass and diversity positively impact soil quality. These microorganisms facilitate the transformation of organic nitrogen (N) and phosphorus (P) found in manures into plant-accessible forms and expedite the decomposition of organic matter (Homyak *et al.*, 2018). The result on Table 2 shows that the T1 value for TBC, TFC and CBC differ significantly from other treatments, T2 value for PSBC differ significantly from other treatment while T3 value for NFBC and Actinobacteria differ significantly from other treatments. The litter soil type influenced the type of microbes that thrived in them. Pig and poultry litter had the highest microbial population of nitrogen fixing bacteria and actinobacteria, poultry litter had the highest microbial population of phosphate solubilizing bacteria while the control had the highest microbial population of bacterial count bacteria, total fungal count and cellulolytic bacteria. These results suggested that N-cycle-related microorganisms were activated by the addition of pig and poultry litter because of their high protein content, possibly through the degradation of organic matters. The result for TFC showed that the value obtained for control was highest compared to other treatment. However, this is in contrast to the fact that fungi thrive in pH tending to acidic level (Meisner *et al.*, 2017).

The highest occurrence of PSB found in the control could be due to the pH level of the soil which tends towards neutral. However, in comparing the litter soils, litter soils from poultry and pig unit had the highest occurrence level of PSB. This could be due to the high level of phosphorus found in poultry and pig litter compared to other livestock.

The highest occurrence of CB was found in the litter soils from poultry and cow unit. This could be due to the high level of cellulolytic bacteria found in the intestine of chicken and rumen of cattle which is passed out through faeces. The CB becomes predominant on the soil where the litters are dumped (Fawole *et al.*, 2016). The highest occurrence of NFB was found in the soils from control, poultry and pig unit. This could be due to the reason that, N-cycle-related microorganisms were activated by the addition of pig and poultry litter because of their high protein content, possibly through the degradation of organic matters (Fawole *et al.*, 2016). The highest occurrence of actinomycetes was found in the litter soil from pig unit. Microorganisms such as bacteria, fungi and actinomycetes play pivotal roles in the degradation of pig faeces and

composting process (Junhua *et al.*, 2022). All these bacteria are sacrosanct to soil-healthiness.

CONCLUSION

This study established soil acidification caused by poorly managed litters from livestock farms which affects soil fertility, microbial population and diversity. Also, there were imbalances in the microbial population and diversity which could be because of the adaptability of the microbes to the various acidic condition.

Conflict of interest

Authors have no conflict of interest to declare

Author contribution

OBM designed and supervised the study. STA carried out the experimental work and drafted the manuscript while author OBM proof-read and corrected the original draft of the manuscript.

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