Bacteriological Investigation of Soil from Different Poultry Farms in Birnin Kebbi Metropolis

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ABSTRACT

This research work is with a view to investigate soil samples from different poultry (Samjol, Real line farm, Ganus and Rafin Atiku) farms in Birnin Kebbi Metropolis. Serial dilution method was used to determine the viable bacteria plate count and biochemical test was used to identify the colonies that developed on the culture medium. The vaiable bacteria plate count indicates that sample (1) from Samjol farm has the highest bacteria load of $1.20x10^{-3}$ cfu while sample(3) from real line poultry farm has the lowest bacteria load of $2.0x10^{-3}$. Ten (10) different kinds of bacteria (Staphylococcus, Escherichia Coli, Klebsiella, Pseudononas, Shigella, Enterobacter, Salmonella, Proteus, Bacillus and Clostridium species) were isolated. The presence of these bacteria in the soil samples from poultry source could pose a potential health risk to the poultry workers, poultry birds sellers and eventually to the public and their presence on the soil could also cause competition with soil microflora resulting in deflection of soil nutrients. Personal hygiene practice of poultry works and birds sellers should be stickly observe to avoid disease outbreak.

INTRODUCTION

Poultry production has been a future of human society for thousands of years (Adewale, 2019). To ensure that it continues to make positive and sustainable contributions to stable human society, it is essential that production and marketing are tailored to local conditions and associated value chains, maximize nutrient cycling and efficient utilization of all products and maintain genetic diversity (Adewale,2019).

Poultry farming is the process of raising different types of domestic birds commercially for the purpose of meat, eggs and feather production (Maher, 2019). The most common and widely raised poultry birds are chicken (both meat and eggs of chicken). The chickens raised for eggs are called layer chickens, and the chickens which are raised for their meat production are called broiler chickens (Maher, 2019).Poultry are used as source of food, income, employment and the products are also used as raw materials for the production of other commodities.

Poultry serve as source of income, employment to the teaming youths, source of organic manure to the crop farmers, food in the form of eggs, meat and also serve as a source of raw materials for the production of other commodities such as the feathers been used for camouflage. Soil is referred to the outer, loose materials of earth surface, a layer distinctly different from the underlying bedrocks, is the key component of natural ecosystem and environmental sustainability depends largely on sustainable ecosystem. Soil supports a complex ecosystem which supports the plants on

the surface and new soils are created from breakdown of rocks and sand. Soil contains a wide range of organisms such as bacteria, actinomycetes, fungi, algae and protozoa. Some soils may contain up to one million species of microbes per gramme, most of these species being unknown, making soil the abundant ecosystem on earth (Asoko, 2021).

Human activities in many parts of the world, e.g., animal production, still impact negatively on the environment and biodiversity. Some of the consequences of man-made pollution include transmission of diseases by water borne pathogens, eutrophication of natural water bodies, accumulation of toxic or recalcitrant chemicals in the soil, destabilization of ecological balance and negative effects on human health (Bager *et al.,* 2017). The continuous drive to increase meat production for the protein needs of the ever increasing world population has some pollution problems attached (Bager *et al.*, 2017).

The poultry industry is one of the largest and fastest growing agro-based industries in the world. This can be attributed to an increasing demand for poultry meat and egg products. However, a major problem facing the poultry industry is the large scale accumulation of wastes including manure and litter which may pose disposal and pollution problems unless environmentally and economically sustainable management technologies are evolved. Most of the produced by the poultry industry is currently applied to agricultural land as a source of nutrients and soil amendment. However environmental pollution, resulting from nutrient and contaminant leaching can occur when poultry litter is applied under soil. Poultry manures are known to harbor human pathogens, culture and molecular-based work has shown that poultry litter is a reservoir for several zoonotic pathogens (Eissler *et al.,*2020) which may contaminate the surroundings (Eissler *et al.,*2020)

Similarly, the physicochemical properties of the soil may become altered, such as the pH, due to the uncontrolled discharge of untreated waste resulting in the loss of certain soil microbes (Bolan *et al.,* 2010). Following the discharge of untreated wastewater into the soil, certain elements (for example, iron, lead, phosphorus, calcium, and zinc) previously absent or present in minute quantities will be introduce into the leading to the magnification of these chemicals and thus altering the physicochemical nature of the soil. Some of these chemicals may be toxic to the microbial, floral and faunal communities of the soil. following the discharge of untreated wastewater into the soil, certain elements (for example, iron, lead, phosphorus, calcium, and zinc) previously absent or present in minute quantities will be introduce into the leading to the magnification of these chemicals and thus altering the physicochemical nature of the soil. Some of these chemicals may be toxic to the microbial, floral and faunal communities of the soil (Boehije et at.,2000).

The discharge of untreated wastes into the environment in Nigeria is still a problem, despite the establishment of Federal Environmental Protection Agency (FEPA) since 2015 (Adeyonu et at., 2021). Different types of layers and broilers are mostly reared in the studied poultries, with their litters been disposed without treatment into nearby land to the poultry. The litters decay with time and mixed with soil, during raining season, the top soil is washed away into water way by erosion within the neighborhood and may affect the whole biological community, including species diversity and contaminant accumulation in the food chain. The aim of this study is to assess the microbiological and physicochemical characteristics of poultry soil samples in Birnin Kebbi.

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and negative effects on human health (Janssen, 2006). The continuous drive to increase meat production for the protein needs of the ever increasing world population has some pollution problems attached (Janssen, 2006).

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STATEMENT OF THE PROBLEM

Although Nigeria is a major producer of so many Agricultural commodities such as Cereals, Aquaculture, Roots and Tubers, Poultry among others, yet, the Country is still reported as an import economy with so many of these commodities being imported from other nations. There are a number of farm surveys on poultry and other livestock farms, but extremely few or scarce survey studies on poultry farm supplies, producers, traders and distributors in Nigeria generally and Kebbi State in particular. If the Poultry farm in the study area is assessed, information provided from the study is likely to be beneficial to both existing and prospective investors in poultry industry by providing ample information to policy makers, existing and prospective investors on poultry actors on the profit realized, technology of production and the different challenges associated with the development of poultry products and suggesting strategies for improvement of poultry products.

As the price of conventional fertilizer soars and interesting alternative sources of crop fertilizer grows, there is an increasing demand for on-farm sources of soil fertility. Integrating livestock and crop production is one channel by which agricultural practitioners can enhance soil fertility using on-farm resources of animal manure andpasture plants. Farmer interest in utilizing pastureraisedchickens (Gallus gallusdomesticus), in particular, to improve soil fertility and enhance crop performance has grown in recent years, as has consumer demand for pastured poultry products. In pastured production, poultry live outdoors and consume pasture plants and grain. Relatively few peer-reviewed articles have been published regarding soil fertility and crop growth under pastured poultry management, yet inferences about the role of poultry on soil fertility can be made from the substantial literature on the fertilizer content values of poultry manure and litter (Kuwornu *et al.,* 2013).

Poultry manure and litter are known effective plant fertilizers. Various estimates give the nutrient concentrations of poultry manure at an average of 5.1% nitrogen (N), 1.9% phosphorus (P) and 2.6% potassium (K) (Kuwornu *et al.,* 2013).

Many field studies suggest that poultry manure and/orlitter application to crop soils can enhance chemical, biological and physical quality, and plant growth. (Janssen,2006) found broiler litter

increased soil carbon (C) and Sequestration in intensive cropping systems at a depth of 0–10 cm. (Janssen, 2006) reported that broiler litter application to cropping systems linearly increased extractable soil P, K, soil cation exchange capacity and soil total Cat 0–15cm in a 3-year study. Land application of poultry litter has also been found comparable to inorganic fertilizer for supplying soil P and increasing forage yield.

Poultry manure and litter have been widely studied because the poultry industry generates high quantities that are frequently applied to crop or pasture fields. Less is known about the effect of utilizing live birds for soil fertilization. Manure deposited by live birds is surface-applied and not tractor-incorporated; although (Janssen, 2006) reported that fecal N and P inputs by free ranging birds corresponded with increased soil N and Plevels. Studies such as these suggest that integrating live poultry into crop agro ecosystems may effect significant changes to soil fertility and yield.

AIM AND OBJECTIVES

The aim of this research work is to investigate the bacteria associated with poultry soil. Through the following objectives:

- To determine the bacteria load present in poultry soil samples collected from different poultry Samjol, Ganus, Rael Line and Rafin Atiku) farms.
- To isolate and characterize the bacteria present in this poultry soil samples.

Justification of the

Studying the entire poultry farms in the metropolis will provide information that will guide existing and prospective investors on which intervention is required in order to enhance poultry products to the market at affordable prices. Researchers and policymakers are also likely to benefit from the outcome of this study.

METHODOLOGY

Sample Collection

Soil Samples were collected from the various poultries in Birnin-kebbi metropolis and transported to Department of Laboratory Science, Waziri Umaru Federal polytechnic, Birnin-kebbi, Kebbi State, for identification or investigation (Carrera *et al.,* 2007).

Sample Preparation

Surface of the soil sample sites was cleared and twenty grammes (20 g) of soil samples in poultry environment were obtained using soil auger at depths of 10cm, samples were collected in cellophane bags that have been previously exposed to ultraviolet radiation for 1 hour and transported to Microbiology Laboratory, School of Science, Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State of Nigeria with one hour of collection. Soil samples used for control were collected from an area devoid of poultry farming or waste in the area (Carrera *et al.,* 2007).

Preparation of Media

All the media used in this research work were prepared in accordance to the manufacturer's instructions as contain in the label on the media containers (Carrera *et al.,* 2007).

Isolation Bacteria

Serial Dilution

Six (6)-fold serial dilutions of each of the poultry soil samples was carried out. Distilled water (5ml) was dispensed into test tubes set up on a rack. Iml of steeped water was collected after stirring to suspend sample and was added to 1ml sterile distilled water to obtain 10^{-1} dilution with the aid of a sterile 1ml pipette. From tube I, 1ml was transferred to tube II to obtain 10^{-2} dilution, the same process was continued until a dilution of 10^{-3} was obtained. 1ml was then removed for culturing on nutrient agar (Maher, 2019).

Determination of morphological characteristics

From the colonies that developed on Nutrient agar, a smear was made on a clean glass slide using sterile wire loop. It was dried and heat fixed. The smear was flooded with crystal violet solution for 60seconds and washed, tipped off and covered with Lugol's iodine for 2 minutes. The stain was then decolourized with acetone and washed off immediately with distilled water. It was then counter stained with safranin for 2 minutes and rinsed with distilled water. The back of the slide was wiped clean; the smear was placed on a draning rack and allowed to air dry. The smear was then viewed under the microscope using oil immersion objectives x 100. The same procedure was repeated for the colonies that developed on the Salmonella-Shigella Agar (Martin *et al.,* 2018).

Biochemical test

Catalase Test

Colonies were picked using sterile wire loop and place on a centre of clean glass slides three (3) drops of hydrogen peroxide was added. The presence of bubble indicate positive while absence indicate negative (Adeyonu *et al.,* 2021).

Coagulase Test

Two colonies were emulsified in 0.5ml of saline contained in a clean stereological tube; 1ml of attracted human plasma was cheeked after 2hours and 4hours of incubation for signs of increased viscosity or complete clothing of the plasma in the absence of increased viscosity of clothing of the plasma after 4hours of incubation, the tube was left over night in the incubation at 37^0c and was observed the following day. Increased viscosity or complete clothing indicates a positive tube coagulase test while absence of viscosity or clothing indicates a negative coagulase test (Martin *et al.,* 2018)

Citrate Test

Simmons citrate agar was inoculated with isolates, using sterile wire loop and incubate for 47hours at 37^0c a deep blue color indicate a positive result (Martin *et al.,* 2018).

Motility test

This contained low concentration of agar (o.2-5%) and motion organisms are able to move away from the line of inoculation through sloppy agar(Martin *et al.,* 2018).

indole test

The organisms were grown in 5ml of peptone waters, nutrient broth for 24hours. After 4 hours of incubation, kowae's indole reagent was added (eight drops). It was shaken gently. A positive reaction was indicated by the development of a red color in the reagent layer above the broth within 1 minute. In negative, the indole reagent is yellow color(Martin *et al.,* 2018).

Oxidase Test

A piece of tissue paper was placed in a clean petridish and 2-3drops of freshly prepared oxidase reagent was added, a colony of the test isolates was picked using a sterile glass rod and smear on the filters paper. Blue-purple colour was observed (Martin *et al.,* 2018).

Results and Discussions

Table1: Shows the results of the microbial load obtained from different Poultry Soil (in cfu)

Sample	Viable Plate Count	cfu
Samjol Venture		
1.	120	$1.20x\ 10^{-3}$
2.	30	$3.0\ x10^{-3}$
3.	80	$8.0x10^{-3}$
4.	65	$6.5x10^{-3}$
5.	55	$5.5x10^{-3}$
Real Line Farm Kawara		
1.	90	$9.0x10^{-3}$
2.	115	$1.15x10^{-3}$
3.	20	$2.0x10^{-3}$
4.	35	$3.5x10^{-3}$
5.	40	$4.0x10^{-3}$
Ganus Farm		
1.	110	$1.10x10^{-3}$
2.	37	$3.7x10^{-3}$
3.	80	$8.0x10^{-3}$
4.	29	$2.9x10^{-3}$
5.	55	$5.5x10^{-3}$
Rafin Atiku Farm		
1.	112	$1.21x10^{-3}$
2.	109	$1.09x10^{-3}$

3.	105	$105x10^{-3}$
4.	99	$9.9x10^{-3}$
5.	65	$6.5x10^{-3}$

KEY: CFU= Colony Forming Unit

Table 2: Shows the results of the bacteria isolates from different poultry soils in Birnin Kebbi Metropolis

Sample	Gram Rxn	Oxid	Cat	Mot	Cit	Ure	Bacterial Isolates
Samjol Venture							
1	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
2	-ve	-ve	+ve	+ve	-ve	-ve	*Escherichia coli*
3	-ve	-ve	+ve	-ve	+ve	+ve	*Klebsiella* Spp
4	-ve	+ve	+ve	+ve	+ve	-ve	*Pseudomonas aeruginosa*
5	-ve	-ve	+ve	-ve	-ve	-ve	*Shigella* Spp.
Real Line Farm Kawara							
1	-ve	-ve	+ve	+ve	+ve	-ve	*Salmonella* Spp
2	-ve	-ve	+ve	+ve	+ve	+ve	*Proteus* Spp
3	-ve	-ve	+ve	+ve	+ve	-ve	Enterobacter
4	+ve	-ve	+ve	-ve	+ve	+ve	*Staphylococcus aureus*
5	-ve	-ve	+ve	-ve	-ve	-ve	*Shigella* Spp.
Ganus Farm							
1	+ve	-ve	-ve	+ve	+ve	-ve	*Clostridium* Spp
2	-ve	-ve	+ve	-ve	+ve	+ve	*Klebsiella* Spp
3	-ve	-ve	+ve	+ve	-ve	-ve	*Escherichia coli*
4	-ve	-ve	+ve	+ve	+ve	-ve	*Salmonella* Spp
5	-ve	-ve	+ve	-ve	-ve	-ve	*Shigella* Spp.

Rafin Atiku Farm							
1	-ve	+ve	+ve	+ve	+ve	-ve	*Pseudomonas aeruginosa*
2	+ve	+ve	+ve	+ve	+ve	-ve	*Bacillus* Spp
3	-ve	-ve	+ve	+ve	-ve	-ve	*Escherichia coli*
4	-ve	-ve	+ve	+ve	+ve	+ve	*Proteus* Spp
5	-ve	-ve	+ve	-ve	-ve	-ve	*Enterobacter aerogenes*

KEY: Oxid = Oxidase Test , Cat =Catalase Test, Mot = Motility Test, Cit =Citrate, Utilization Test, Ure =Urease Test , -ve = Negative , +ve = Positive

Discussion

The bacteriological investigation of poultry soils from different poultry farms carried out to ascertain the types of bacteria that could be associated with the poultry soils. Serial dilution method was used to determine the viable bacteria plate count and the colonies that develop from the medium used were Gram stained and subjected to biochemical test to confirm the species of the microorganism. The poultry farms are samjol Ventures (A), Real Line Farm Kawara (B), Ganues Farm (C) and Rafin Atiku (D) located in Birnin Kebbi metropolis.

The viable bacteria plate count from poultry (A) farm soil samples ranges from $3.0x10^{-3}$ to $1.20x10^{-3}$cfu, that of poultry (B) farm soil sample ranges from $2.0x10^{-3}$ to $1.15x10^{-3}$ cfu, that of poultry (C) farm soil sample ranges from $2.9x10^{-3}$ to $1.1x10^{-3}$ cfu, while that ofpoultry (D) farm soil sample ranges from $6.5x10^{-3}$ to $1.21x10^{-3}$ cfu. These results suggest that sample(1) from (A) Farm has the highest bacteria load of $1.20x10^{-3}$ cfu while Sample (3) from (B) Farm has the lowest bacteria load of $2.0x10^{-3}$ cfu.

This finding confirms the findings of Gebremedlin, (2015), who reported heavy loads of microbes found in the poultry soil could be associated with poultry dumps that act as nutrients that enable the microorganism to strive well in poultry soil environment.

Ten (10) different kinds of bacteria (*Staphylococcus, Escherichia coli, Klebsiella, Pseudomonas, Shigella, Enterobacter, Salmonella, Proteus, Bacillus and Clostridium*) species were isolated and most of these bacteria are pathogenic and their presence in the poultry soil could pose a public health risk. The presence of Staphylococcus species like *S. epidermidis* could cause skin injection, *S aureus* could cause impetigo, wounds, furunculitis, carbuncle while *S. saprophyticus* could cause urinary infection in young women and this *Staphylocossus* species is encountered in the poultry soil. *Salmonella, Klebsiella, Shigella, Enterobacter*, *Pseudomona*s and Proteus are all gram negative bacteria encountered in the soil samples from poultry. The infection cause by these bacteria could be very severe due to the nature of the Gain negative bacteria cell of the *pepidoglycan* that has additional periplasmic space and Lipopolysaccharide layer in addition to the alternating subunits of the peptidoglycan. The infection or disease cause by these Gram negative bacteria could be typhoid, dysentery to gastrointestinal disorders to enteric fever. Their presence in the poultry soil could pose a potential health risk to poultry workers and buyers or sellers of poultry birds. Bacillus and Clostridium species were also encountered in the poultry soil. They are Gram positive rod shape bacteria and are spore formers. They form spores that help them to withstand adverse environmental conditions. They are also associated with food born-illness or

food poisoning. Their presence in the poultry soil should be of public health concern. Also the presence of these bacteria species in the poultry soil could result in serious competition for nutrients with soil microorganisms and the competition may laid to the depletion of soil nutrients, thereby resulting to the death of soil microbes from starvation. (Tijjani *et al.,* 2012). Reported that poultry manures (soil are knows to harbor human pathogens. This research work as clearly shows that the poultry soils harbor different kinds of pathogens. Therefore, personal hygiene practice of poultry workers and sellers of poultry birds should be observed.

Conclusion

The bacteriological investigation of soil samples from different poultry (Samjol, Realine, Ganus and Rafin Atiku) farms were carried out using serial dilution method and biochemical test. The vaiable bacteria plate count indicates that sample (1) from Samjol farm has the highest bacteria load of $1.20x10^{-3}$ cfu while sample (3) from real line poultry farm has the lowest bacteria load of $2.0x10^{-3}$ cfu. Ten (10) different kinds of bacteria (*Staphylococcus, Escherichia coli, Klebsiella, Pseudomonas, Shigella, Enterobacter, Salmonella, Proteus, Bacillus and Clostridium*) species were isolated. The presence of these bacteria in the soil samples from poultry source could pose a potential health risk to the poultry workers, poultry bird's sellers and eventually to the publi

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