Microbiological and Physicochemical Assessment of Poultry Soil Samples in Akure Metropolis, Nigeria

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Abstract— Human activities such as animal production in many parts of the world, still impact negatively on the environment and biodiversity. This study was carried out to assess the microbiological and physicochemical parameters of poultry soil samples. Soil samples were collected from different poultry in Akure metropolis and soil samples from Federal University of Technology, Akure (FUTA) environment as control. Microbiological and physicochemical analyses were carried out using standard methods. The mean total viable bacterial count of poultry soil ranged from 9.02±0.511×10⁵cfu/g in sample site A to $11.2\pm0.021\times10^{3}$ cfu/g in sample site B and there were significant difference (p<0.05) between the bacterial count of poultry soil and control, mean highest fungal load is 6.05±0.301× 10³ sfu/g. Bacteria isolated were Escherichia coli, Aeromonas hydrophila ,Bacillus subtilis, Bacillus cereus, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Streptococcus faecium. Escherichia coli had the highest occurrence of 23.08% while Aeromonas hydrophila had the lowest occurrence of 5.13%. Fungi isolated were Aspergillus flavus, Aspergillus niger, Aspergillus fumigates and Saccharomyces species. Aspergillus niger 46.67% had the highest occurrence while Saccharomyces species had the lowest occurrence of 13.33%. Physiochemical analysis showed that poultry soil had mean pH, temperature and Organic Carbon of 7.92±0.34, 34±0.04°C and 14.88±0.56% respectively, Organic Matter is 5.50±0.61%, Nitrogen 1.27±0.03 mg/g, Phosphorus58.92±0.01 mg/g, Potassium55.48±0.23 mg/g, Sodium 41.77±0.91mg/g, Calcium 28.70±0.24mg/g, Magnesium 20.65±0.32 mg/g and Cation exchange capacity146.60±0.11%. The results obtained calls for proper discharge of poultry waste into the environment to prevent transmission of diseases by water borne pathogens.

Keywords—Akure, Microbiological, Physicochemical, poultry, soil.

I. INTRODUCTION

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 (Arotupin *et al.*, 2013; Obasi *et al.*, 2013).

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 (Amisu *et al.*, 2003). The continuous drive to increase meat production for the protein needs of the ever increasing world population has some pollution problems attached (Eze *et al.*, 2013).

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 litter 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 (Bolan *et al.*, 2010) which may contaminate the surroundings.

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 (Rabah et al., 2010). Tortora et al. (2007), reported that

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.

The discharge of untreated wastes into the environment in Nigeria is still a problem, despite the establishment of Federal Environmental Protection Agency (FEPA) since 1998 (Adeyemo, 2003). 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 Akure.

II. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at the Department of Microbiology Laboratory, School of Science, Federal University of Technology, Akure (FUTA), Nigeria. Akure is the largest city and capital of Ondo State, located in south-west Nigeria. Akure lies about 70°15 north of the equator and 50°15 east Meridian. The city has a population of 588,000 which is 0.305% of Nigeria population based on 2006 national population census, the people are of Yoruba ethnic group and are situated in the tropic rainforest.

2.2 Sample Collection

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, Federal University of Technology, Akure, Nigeria with one hour of collection. Soil samples used for control were collected from an area devoid of poultry farming or waste in FUTA area.

2.3 Microbiological Analysis of the Soil samples

Nutrient agar (NA) and Potato Dextrose agar (PDA) medium was prepared according to manufacturer's instruction and sterilized. One gramme of each soil sample from different poultry soil and FUTA soil (control) was diluted serially until fifth dilutions. An aliquot of 0.1ml from the fourth and fifth dilution was inoculated on the freshly prepared media using pour plate method, incubated at 37°C for 24 hours for NA and 28±2°C for 3 to 7days and observed for growth. Colonies of bacteria and spores of fungi were recorded as colony forming units per gram of soil (cfu/g) and spore forming units per gram of soil. Isolates were subcultured repeatedly to obtain pure cultures. The pure cultures were characterised using standard microbiological techniques (Oyeleke and Okusanmi, 2008b; Cheesbrough, 2006).

The tests employed include colonial, morphological characteristics, gram stain, motility, catalase, methyl red, Voges-Proskaeur, indole production, urease activity, H_2S and gas production, citrate utilization, glucose, sucrose, and lactose utilization tests. The fungal isolates were identified according to method of Dunca *et al.*, (2004) and Oyeleke and Okusanmi (2008b) based on the colour of aerial hyphae and substrate mycelium, arrangement of hyphae, conidial arrangement as well as morphology.

2.4 Determination of Physicochemical properties of the Soil samples

The physicochemical properties of the soil samples were determined. The colour and particle sizes of the soils were determined using standard methods. The pH was measured using pH meter standardized at pH 7.0 using appropriate buffers (Ibitoye, 2006). The moisture content of each soil sample was determined by drying 10grammes of the soil in an oven at 80°C until a constant weight was reached and the percentage moisture content was calculated. The organic carbon content was determined using the Walkey-Black wet oxidation method as described by Ibitoye (2006). Available phosphorus, exchangeable magnesium, calcium, sodium and potassium ion concentration were determined using standard methods (AOAC, 1990; APHA, 2005). Total nitrogen was measured using the Macrokjeldahl digestion method (Heads, 1992).

2.5 Data analysis

Statistical analysis of data was done using one way analysis of variance (ANOVA) and Means was compared by Duncan's New Multiple Range test using SPSS version 20.0. Difference was considered significant at P < 0.05.

III. RESULTS

The microbiological and physicochemical analysis results are represented in Table 1, 2 and 3.

Table 1 shows the total mean viable count of bacteria and fungi in Poultry soil and FUTA soil (control). The mean total viable bacterial count of poultry soil ranged from $9.02\pm0.511\times10^5$ cfu/g in sample site A to $11.2\pm0.021\times10^5$ cfu/g in sample site B and there were significant difference (p<0.05) between the bacterial count of poultry soil and control. Bacterial count of the control soil sample was also observed to be smaller than poultry soil. However, there was no significant difference in fungal load observed in all poultry soil samples and control.

TABLE 1
TOTAL VIABLE COUNTS OF BACTERIA AND FUNGI IN POULTRY SOIL AND FUTA SOIL (CONTROL).

	Soil sample					
Microorganism						
	A	В	С	FUTA soil (Control)		
Bacteria (cfu/g×10 ⁵)	10.2±0.721°	11.2±0.021°	9.02±0.511 ^b	1.7±0.54 ^a		
Fungi (sfu/g× 10 ³)	6.05±0.301 ^a	5.05±0.902 ^a	5.47±0.501 ^a	5.15±0.501 ^a		

Counts represent means of triplicate samples \pm standard error, values in the same row with the same superscript are not significantly different at (p < 0.05) according to Duncan's New Multiple Range test. **Key:** cfu/g: colony forming units per gramme, sfu/g: spore forming units per gramme.

Table 2 shows the microorganisms isolated and their percentage occurrence. Bacteria isolated were *Escherichia coli*, *Aeromonas hydrophila*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus faecium*. *Escherichia coli* 23.08% had the highest occurrence followed by *Bacillus cereus*17.95% while *Aeromonas hydrophila* had the lowest occurrence of 5.13%. Fungi isolated were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigates* and *Saccharomyces* species. *Aspergillus niger* had the highest occurrence of 46.67% while *Saccharomyces* species had the lowest occurrence of 13.33%.

The physiochemical parameters of poultry soil and control soil samples shown in Table 3. The mean value of physiochemical parameters of poultry soil for pH, temperature and Organic Carbon are 7.92 ± 0.34 , $34\pm0.04^{\circ}$ C and $14.88\pm0.56\%$ respectively, value of Organic Matter is $5.50\pm0.61\%$, Nitrogen 1.27 ± 0.03 mg/g, Phosphorus 58.92 ± 0.01 mg/g, Potassium 55.48 ± 0.23 mg/g, Sodium 41.77 ± 0.91 mg/g, Calcium 28.70 ± 0.24 mg/g, Magnesium 20.65 ± 0.32 mg/g and Cation exchange capacity $146.60\pm0.11\%$. However, the values of physiochemical parameters of control were lower compared to the values obtained in poultry soil.

TABLE 2
MICROORGANISMS ISOLATED FROM POULTRY SOILAND THEIR PERCENTAGE (%) OCCURRENCE

Isolates	Number of isolates	(%) Occurrence	
Bacteria			
Aeromonas hydrophila	2	5.13	
Bacilllus subtilis	5	12.82	
Bacillus cereus	7	17.95	
Escherichia coli	9	23.08	
Klebsiella pneumonia	3	7.69	
Pseudomonas aeruginosa	3	7.69	
Salmonella typhi	3	7.69	
Staphylococcus aureus	4	10.26	
Streptococcus faecium	3	7.69	
Total	39	100	
Fungi			
Aspergillus flavus	3	20	
Aspergillus niger	7	46.67	
Aspergillus fumigates	3	20	
Saccharomyces species	$\overline{2}$	13.33	
Total	15	100	

TABLE 3
PHYSICOCHEMICAL PARAMETERS OF POULTRY AND FUTA SOIL (CONTROL).

Sample	Poultry soil	FUTA soil(control)	FME limit	DPR limit
рН	7.92±0.34	7.92±0.33	6.00-9.00	6.0-8.5
Temperature (°C)	34±0.04	33±0.91	40.00	30
Organic Carbon (%)	14.88±0.56	0.56±0.21	NA	NA
Organic Matter (%)	5.50±0.61	0.96±0.35	NA	NA
Nitrogen (mg/g)	1.27±0.03	0.90±0.30	NA	NA
Phosphorus (mg/g)	58.92±0.01	2.86±0.05	5.0	NA
Potassium (mg/g)	55.48±0.23	0.10 ± 0.04	NA	NA
Sodium (mg/g)	41.77±0.91	0.08 ± 0.26	NA	NA
Calcium (mg/g)	28.70±0.24	0.80±0.33	0.2	0.2
Magnesium (mg/g)	20.65±0.32	0.50±0.14	0.2	0.2
Cation exchange capacity (%)	146.60±0.11	1.48±0.04	NA	NA

Values are mean of triplicate ± standard error. FME: Federal Ministry of Environment; NA: not available; DPR= Department of Petroleum Resources.

IV. DISCUSSION

The high counts of both bacteria and fungi obtained indicated that the poultry soil had a high microbial load than the FUTA (control) soil. Also, it revealed a significant difference (p<0.05) between the counts of bacteria in the poultry soil compared to the FUTA (control) soil. This was because the poultry soil has been contaminated with poultry droppings, poultry droppings has chemical composition that supports the growth of billions of microorganisms (Adegunloye, 2006) but are not available in FUTA (control) soil. Also, high bacterial load may be attributed to destabilization of soil ecological balance as a result of the discharged of untreated poultry droppings that habours bacteria which are normal intestinal floral of poultry birds. The result is in line with the reports of Adesemonye *et al.*, (2006) and Rabah *et al.*, (2010) who reported high microbial load in contaminated soil. These microorganisms can contaminate surface and underground water which will pose a serious health hazard.

The microorganisms isolated were bacteria; Aeromonas hydrophila, Bacillus subtilis, Bacillus cereus, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Streptococcus faecium and fungi; Aspergillusflavus, Aspergillusniger, Aspergillus fumigates and Saccharomyces species. The presence of Bacillus subtilis and Bacillus cereus observed in the poultry soil may not be surprising as these organisms are indigenous to soil environment and are known to persist in such environment (Atlas and Bartha, 2007), it was also isolated from poultry droppings by Adegunloye, (2006) therefore contamination by poultry litters may have contributed to the presence of Bacillus subtilis and Bacillus cereus as well. However, the presence of Aeromonas hydrophila, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Streptococcus faecium in the poultry soil may be as a result of large quantity of animal poultry droppings deposited in the surrounding soil. Similar findings were reported by Ezeronye and Ubalua, (2005); Bala (2006) and Rabah et al. (2010) in contaminated soil. These bacteria have also been isolated from poultry droppings by Andrew et al. (2007), Ngodigha and Owen (2009) and Omojowo and omojasola (2013). It is also an indication of recent faecal pollution. The fungal isolates were also soil-inhabiting microorganisms (Atlas and Bartha, 2007) as well as common spoilage organisms associated with poultry industry, Aspergilli are predominantly storage fungi and are present in most stored poultry feeds, Aspergillus species also cause different type of diseases in poultry according to Adegunloye (2006), who isolated Aspergillus species from poultry droppings stated that Aspergillus species are mostly associated with poultry and can cause aflatoxicosis or aspergillosis. The presence of these organisms is a pointer to possible pollution and may have an effect on the soil ecological balance.

The changes in the physicochemical were as a consequence of the buildup in the breakdown of poultry droppings. The mean values of physicochemical parameters were higher in the poultry soil samples than in control, Federal Ministry of Environment limit and Department of Petroleum Resources limit for discharge into the environment. However pH and temperature were within the range. The changes must have been caused by discharged of poultry droppings which is very reach in many nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), trace elements, such as Copper, Zinc, Arsenic and organic matters (Bolan *et al.*, 2010) into poultry environment. High Cation exchange capacity observed in poultry soil compared to control is a reflection of increased soil conditions with regard to the agricultural potential of poultry

soils. Such changes in soil condition are favorable since they promote the increase of organic colloids and humic acids and better nutrient availability (Eze *et al.*, 2013). Poultry litter is very good organic manure, It has historically been used as a source of plant nutrients and as a soil amendment for vegetable crop production (George *et al.*, 2009).

V. CONCLUSION

High microbial load in poultry soil observed in this study suggest that poultry soil are heavily contaminated with poultry waste which encourage the proliferation of microorganisms and this can alter soil biodiversity. The presence of human pathogenic bacteria in poultry soil shows that soil in poultry environment is a public health concern as water can runoff the surface soil and passed in to surface and underground water. This will expose those living in poultry environment to water borne bacterial pathogens. However, the physicochemical parameters; pH, temperature, high mineral content and cation exchange capacity shows that poultry soil will be very good for agricultural purposes. Therefore, poultry farmers need to be aware of the potential health risk of discharging poultry waste into their environment without treatment.

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