

## EFFECTS OF ANIMAL MANURES ON ENZYMES ACTIVITIES AND PHYSICO-CHEMICAL PROPERTIES OF A DEGRADED HUMID ULTISOL

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### ABSTRACT

*Application of animal manures for soil amendment plays a major role in the improvement of soil properties and enzymatic activities of a degraded Ultisol. This study assessed the effects of poultry manure (PM) and swine manure (SM) on the activities of catalase and urease enzymes and some soil properties. The PM and SM were applied at the rate of 30 t ha<sup>-1</sup> each on experimental plots arranged in a randomized complete block design with three replicates. Soil samples were collected at day 0, 14, 28, 42, 56, 70 and 84 from 0-15 and 15-30 cm depths and analyzed for catalase and urease enzymes and some soil properties using standard procedures. The results showed increase in soil pH (in H<sub>2</sub>O) from 4.0 to 5.4 following manure application. At 0-15 cm soil depth, PM and SM recorded 28.1 and 28.8% increases in soil pH (in H<sub>2</sub>O), respectively. Soil organic carbon was highest (2.6 g kg<sup>-1</sup>) at 0-15 cm depth for soil amended with SM while the lowest value of 1.1 g kg<sup>-1</sup> was obtained at 15-30 cm depth for soil unamended with SM. In PM-amended soil, catalase activities ranged from 1.32 to 6.77 mg g<sup>-1</sup> while its activities in SM-treated soil significantly ( $p < 0.05$ ) varied between 1.55 and 8.11 mg g<sup>-1</sup>. Urease showed ranges of 0.72-3.90 mg g<sup>-1</sup> and 0.96-4.71 mg g<sup>-1</sup> in PM-amended and SM-treated soils, respectively. The results uphold that animal manures improve soil properties and are enzymatically controlled.*

**Key words:** catalase, urease, soil amendments, soil properties

### INTRODUCTION

Soil quality is the ability of the soil to perform functions required for the need of the people and the environment. These functions are well-defined by the soil physico-chemical and biological properties of soil. Soil is vital in organic matter decomposition, nutrient cycling and water retention and release. Soil amendments such as animal manures contribute to increases in biological activity as well as to improvements in fertility of the soil. Animal manures play critical role in the soil such as improvement of organic carbon and organic matter (Adebola *et al.*, 2017), amelioration of soil acidity (Nwite *et al.*, 2012; Onunwa *et al.*, 2021), increased release of nutrients (Mohamed *et al.*, 2010; Adubasim *et al.*, 2018), and enhancement of soil microbial and enzyme activities (Afangide *et al.*, 2020; Ogumba *et al.*, 2020). Animal manures serve as substrate for microorganisms which are the main source of soil enzymes that ensure organic matter breakdown (Speir and Ross, 1978; Green *et al.*, 2007). The activities of enzymes are impacted on by environmental factors such as temperature, soil pH and depth of soil (Peterson and Anderson, 2005). The enzymes involved in decomposition of animal manures are bioindicators and are regarded as a measure of soil quality because of their relation with the cycling of soil minerals and biochemicals in soils (Buturuga *et al.*, 2016).

Catalase (H<sub>2</sub>O<sub>2</sub> oxidoreductase, EC 1.11.1.6) is an enzyme capable of performing critical role in soil fertility. Its activities are connected to the metabolic action of aerobic microorganisms (Shiyin *et al.*, 2004; Trasar-Cepeda *et al.*, 2007). Catalase is an anti-oxidant enzyme that is capable of breaking down of H<sub>2</sub>O<sub>2</sub> into water and oxygen without causing free radicals. It is highly stable and demonstrates positive relationship with soil organic carbon; so, it decreases with soil depth (Alef and Nannipieri, 1995). Urease (urease amidohydrolase, EC 3.5.1.5) hydrolyzes urea fertilizers enzymatically into NH<sub>3</sub> and CO<sub>2</sub> that lead to increases in soil pH (Andrews *et al.*, 1989), causing loss of N to the atmosphere because of volatilization of urea N as ammonia (Simpson *et al.*, 1984). However, the activity of urease in soil is of utmost importance due to its ability to regulate the supply of N to plants after urea application.

Information on catalase and urease activities in humid tropical soils of Uyo amended with animal manures has not been completely elucidated. It is against this backdrop that this study was carried. Therefore, the objectives of this study were to assess the effect of poultry (PM) and swine manures (SW) on catalase and urease activities throughout out the period of decomposition of animal manures and the influence of animal manures on physico-chemical properties of soils of the study area.

## MATERIALS AND METHODS

### Study Area

The study was carried out in Uyo, southeastern Nigeria. The area lies between latitude 5° 01' 05" and 5° 01' 10" N and longitude 7° 59' 50" and 7° 59' 55" E. The area lies within the humid tropics characterized by high temperature with annual range of 26 to 31°C, while rainfall is averaging between 2000 to 2500 mm. Relative humidity is about 75%. The rainfall pattern is bimodal with peak in July and a short period of dry spell in August known as August break. Degraded Ultisol having no apparent spatial differences in soil properties and has been under cultivation for more than 20 years was used for the study. The soil Typic paleudults derived from coastal plain sand (Orajiaka, 1975).

### Field Study

The experimental field selected for the study was cleared, ploughed, harrowed and mapped out into experimental plots. Each plot size was 3 × 3 m separated by 1 m path arranged in a randomized complete block design with three replicates. The treatments were cured poultry manure (PM) from deep litter broiler pen and swine manure (SM) obtained from the University of Uyo Teaching and Research Farm, Nigeria. The treatments application rate was 30 t ha<sup>-1</sup> and the treatments were allowed to decompose for a period of ninety days. Using soil auger and sterilized polyethene bag, soil samples were collected from depths 0-15 cm and 15-30 cm during the decomposition time at day 0, 14, 28, 42, 56, 70 and 84. A total of 12 samples were obtained in every week, summing to a total of 84 samples for enzymatic analyses. Soil samples were also obtained for physico-chemical analyses before treatments were applied on the soil and after 90 days of decomposition of the treatments.

### Laboratory Analyses

The soil samples were air-dried, sieved through a 2-mm sieve and sent to laboratory for analyses. The analyses carried out were particle size distribution as described by Gee and Or (2002); soil pH, measured using glass electrode (Thomas, 1996); organic carbon determined using modified Walkley-Black procedure (Nelson and Sommers, 1996); total nitrogen determined by Macro-Kjedahl method (Bremner, 1996); available phosphorus determined using calorimetric method (Olson and Sommers, 1982); and effective cation exchange capacity and exchangeable acidity, following the procedures described by Thomas (1996). The manures were air-dried and hammer-milled to obtain particle less than 1.5 mm in size. Manures were characterized by the method proposed by Kaira and Maynard (1991).

### Determination of Urease Activity

A 5 g moist soil sample mixed with 1 ml of toluene in a 50 ml Erlenmeyer flask was allowed to remain for 15 min. (Gu *et al.*, 2009). Then 10 ml of 1% urea solution and 20 ml citrate buffer (pH 6.7) were added, thoroughly mixed, and kept in an incubator

for at 37°C for 24 h. The soil was filtered through Whatman 42 filter paper after it was incubated. The 50 ml test tubes used were filled with 3 ml aliquot and 4 ml sodium phenol and 3 ml sodium hypochlorite were added. After 20 min., the final volume was made to 50 ml using distilled water, the absorbance of the release urease was measured when the colour was developed at room temperature at 578 nm and the result expressed as mg NH<sub>4</sub>-N in Mg g<sup>-1</sup> soil.

### Determination of Catalase Activity

A 3-g moist soil sample was measured and added to a 50 ml Erlenmeyer flask, 40 ml of distilled water, 5 ml of 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 5 ml of KMnO<sub>4</sub> were added. Thereafter, 10 ml phosphate buffer (pH 6.5) were added and shaken for 20 min. with the help of an end-to-end shaker as described by Cohen *et al.* (1970). The reaction was terminated by an addition of 10 ml of 3NH<sub>2</sub>SO<sub>4</sub> after it was shaken. Catalase was measured calorimetrically at 480 nm and the result recorded as mg H<sub>2</sub>O<sub>2</sub> g<sup>-1</sup> of soil.

### Statistical Analysis

Data on urease, catalase and soil physicochemical properties were subjected to analysis of variance using the software package (SAS, 1999). Treatment means that differed significantly at  $p \leq 0.05$  were separated using the Duncan's new multiple range test.

## RESULTS AND DISCUSSION

### Manure Characterization

Table 1 shows chemical characteristics of poultry and swine manures. Poultry manure was higher in pH, organic matter and available phosphorus. Higher level of chemical properties of poultry manure such as pH, available phosphorus, and organic matter may be due to variations in feed given to poultry, mineral supplements and bedding materials. It has been observed that fluctuation in animal diet, age, type, environment and productivity may cause differences in chemical properties of poultry manures (Afangide *et al.*, 2020). Swine manure recorded higher amount of total nitrogen, organic carbon and CN ratio. By this higher CN ratio, the rate of mineralization is expected to be lower in the soil amended with swine manure relative to the soil amended with poultry manure (Azam, 2002; Uzoh *et al.*, 2015). The soil physicochemical properties of the control site and manure amended soil are shown in Tables 2 and 3.

**Table 1:** Some chemical characteristics of the animal manures used for the study

Properties	Poultry manure	Swine manure
Total nitrogen (g kg <sup>-1</sup> )	44.2	52.4
Organic matter (g kg <sup>-1</sup> )	724.3	648
Organic carbon (g kg <sup>-1</sup> )	290	373
Available phosphorus (mg kg <sup>-1</sup> )	19.2	15.0
C/N	5.3	7.8
pH-H <sub>2</sub> O	7.58	6.62

**Table 2:** Physico-chemical properties of the soils of the study area before application of animal manure

	0-15 cm P <sub>1</sub>	15-30 cm P <sub>1</sub>	0-15 cm S <sub>1</sub>	15-30 cm S <sub>1</sub>
Sand (g kg <sup>-1</sup> )	862b	877a	855b	852a
Silt (g kg <sup>-1</sup> )	52.3acd	36.0abc	62.0ab	49.0abc
Clay (g kg <sup>-1</sup> )	85.7c	87.3bc	83.0c	99.0a
pH in H <sub>2</sub> O	4.2c	4.1d	4.1c	4.0c
Soil organic carbon, SOC (g kg <sup>-1</sup> )	1.4b	1.1b	1.3ab	1.1b
Total Nitrogen, N (g kg <sup>-1</sup> )	0.1bc	0.1cd	0.1a	0.0cd
Available phosphorus, P (mg kg <sup>-1</sup> )	43.3bcd	34.7d	42.0bc	35.0ac
Effective cation exchange capacity, ECEC (cmol kg <sup>-1</sup> )	10.4c	10.2c	10.3c	9.8b

Mean values with the same letter (s) within the rows are not significantly different from one another at  $p < 0.05$ .

P<sub>1</sub> and S<sub>1</sub> represent control soil (untreated) with poultry and swine manures, respectively.

**Table 3:** Physico-chemical properties of the soils of the study area 90 days after application of animal manures

	0-15 cm P <sub>2</sub>	15-30 cm P <sub>2</sub>	0-15 cm S <sub>2</sub>	15-30 cm S <sub>2</sub>
Sand (g kg <sup>-1</sup> )	846a	862a	840b	842b
Silt (g kg <sup>-1</sup> )	48.0d	22.0cd	42.0acd	38.0d
Clay (g kg <sup>-1</sup> )	106ab	116abc	118cd	120ab
pH in H <sub>2</sub> O	5.4a	4.8a	5.2a	4.7ab
Soil organic carbon, SOC (g kg <sup>-1</sup> )	2.3a	2.0a	2.6a	2.0a
Total nitrogen, N (g kg <sup>-1</sup> )	0.1a	0.1b	0.1a	0.1bc
Available phosphorus, P (mg kg <sup>-1</sup> )	72.0b	65.0ab	68.0ab	62.0b
Effective cation exchange capacity, ECEC (cmol kg <sup>-1</sup> )	18.3a	13.9b	16.8a	13.4b

Mean values with the same letter (s) within the rows are not significantly different from one another at  $p < 0.05$ .

P<sub>2</sub> and S<sub>2</sub> represent control soil (untreated) with poultry and swine manures, respectively.

Sand, silt and clay contents showed values in the ranges of 840-877, 22.0-62.0 and 83.0-120.0 g kg<sup>-1</sup>, respectively. The dominance of sand-sized particle may be due to abundance of rainfall in the area which promotes erosion menace (Abraham, 2010). Consequently, the increase in clay fractions with soil depth could be attributed to illuviation of clay and its translocation, which is accelerated by increase in runoff impact due to high rainfall (Obi *et al.*, 2009). Soil pH in H<sub>2</sub>O was generally acidic 4.0-5.4. This can be attributed to acidic nature of the parent material (Ndukwu *et al.*, 2012). Poultry manure treated soil recorded 28.1% increase of soil pH at the 0-15 cm soil depth while swine manure treated soil increased by 28.8% also at 0-15 cm soil depth. Similarly, soil pH of poultry manure amended plots increased by 18.1% at 15-30 cm soil depth while swine manure amended soil recorded 17.9% increase at 15-30 cm soil depth following manure application. Low values of soil pH less than 5.5 may be due to high amount of rainfall in the region which promotes leaching of basic cations from the soil (Uzoho *et al.*, 2007). Some chemical properties of the soil including soil organic carbon, total N, available P and effective cation exchange capacity were low and decreased significantly ( $p < 0.05$ ) with increased soil depth. Total N recorded highest value at the 0-15 cm soil depth treated with swine manure. Also, soil organic carbon was highest at the 0-15 cm soil depth amended with swine manure. These may be ascribable to higher level of animal manures on the surface layer soil (Zhijin *et al.*, 2013).

Table 4 shows dynamics of catalase and urease enzymes and their variations across animal manures. Dynamics of catalase activities ranged from 1.32 to 6.77 mg g<sup>-1</sup> in amended soil with poultry manure while its activities in swine manure treated soil varied between 1.558.11 mg g<sup>-1</sup>. Catalase was highest in the soil at 0-15 cm soil depth at week 2 of decomposition. This may be attributable to higher microbial abundance in the surface layer of the soil since the deepest layers usually have reduced number of microbial cell and subsequently, enzyme level showed decreasing trend. But due to humid rainfall characteristic of the area which promotes high leaching and mineralization of the animal manures, 15-30 cm soil depth recorded a significant increase in enzymes production. Similar observations were made by Chang *et al.* (2007) who reported that catalase production is a function of organic matter level of the soil. Catalase activities were higher in poultry manure treated soil than swine manure treated soil. The increase of catalase may be associated with a decrease in the nitrogen level of poultry manure when compared to swine manure during characterization (Table 1). This in is line with Xia *et al.* (2017) who reported that, increase in nitrogen level of animal manures will lead to a decrease and a negative relationship with catalase activity. Urease ranged from 0.72 to 3.90 mg g<sup>-1</sup> in amended soil with poultry manure whereas in soil amended with swine manure, urease varied between 0.96 and 4.71 mg g<sup>-1</sup>. The amount of urease was higher in swine manure amended soil when related to

**Table 4:** Changes in catalase and urease activity in topsoil and subsoil treated with poultry and swine manures

Duration	Catalase (mg g <sup>-1</sup> )		Urease (mg g <sup>-1</sup> )	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Poultry manure treated soil				
Week 0	1.48a	1.32a	1.08b	0.72b
Week 2	4.42a	1.95b	2.50a	2.24a
Week 4	3.38b	5.96ab	2.15a	2.52ab
Week 6	2.90a	6.77ab	2.14a	3.90b
Week 8	2.43b	3.92ab	2.04a	2.51a
Week 10	2.33ab	2.89a	1.76b	1.97b
Week 12	1.88b	2.50ab	1.29a	1.21a
Swine manure treated soil				
Week 0	1.72b	1.55b	1.08a	0.96b
Week 2	5.25a	2.10b	4.05a	1.89b
Week 4	4.26b	7.14ab	3.07a	4.08a
Week 6	4.04a	8.11ab	2.65b	4.71a
Week 8	2.61b	5.28ab	1.53a	2.02a
Week 10	1.82b	2.65b	1.34a	2.14b
Week 12	1.79b	3.00ab	1.16a	1.69a

Mean values with the same letter (s) within the rows are not significantly different from one another at 5% probability level

poultry manure amended soil. The observed increase may be largely due to high amount of N in pig manure than poultry manure (see Table 1), since increase in N often leads to increase in urease activity (Kilzilkaya and Elberli, 2008).

### CONCLUSION

This research showed that animal manures improved some chemical properties of the soil such as pH, organic carbon and total nitrogen were improved after manure application. There was elevated amount of catalase and urease activities at the second week of manure decomposition (14 days after application) of poultry and swine manures while sub-surface layer of the soil produced the highest amount of catalase and urease at the sixth week of decomposition (42 days after application) of the manures. Urease activity was highest in swine manure amended soil whereas catalase activity was highest in poultry manure amended soil. Based on this finding it is better to allow animal manures to decompose at least two weeks prior to planting of crops.

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