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RESEARCH ARTICLE

DYNAMICS OF PHOSPHATASE ENZYME AND MICROBIAL PROPERTIES IN A DEGRADED ULTISOL AMENDED WITH ANIMAL MANURES

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ABSTRACT

Application of animal manures influences enzymatic activities and microbial dynamics in soils. Poultry manure (PM) and swine manures (SM) were applied at the rate of 30 t ha^{-1} each. The experiment was arranged in a randomized complete block design with three replicates. Soils were sampled at day 0, 14, 28, 42, 56, 70 and 84 from 0-15 cm and 15-30 cm depths. In both the PM and SM amended soils, significant increase in soil pH was observed. In PM treated soil, alkaline phosphatase significantly increased from 0.38 Mg g^{-1} to 4.94 Mg g^{-1} whereas in SM treated soil, it increased from 1.21 Mg g^{-1} to 4.80 Mg g^{-1}. Acid phosphatase significantly increased from 0.42 Mg g^{-1} to 3.02 Mg g^{-1} in PM amended soil while increases from 0.11 Mg g^{-1} to 2.38 Mg g^{-1} were observed in SM amended soil. The application of PM and SM increased Total Heterotrophic Bacterial Count significantly from $1.27x10^4$ Cfu g^{-1} to $8.63x10^7$ Cfu g^{-1} and from $1.40x10^4$ Cfu g^1 to $8.10x10^7$ Cfu g^{-1}, respectively. Total Fungal Count significantly increased from $1.63x10^3$ Cfu g^{-1} to $4.67x10^6$ Cfu g^{-1} and from $2.00x10^3$ Cfu g^{-1} to $5.67x10^6$ Cfu g^{-1} in PM and SM amended soils, respectively.

KEYWORDS

Enzymatic activities, Soil properties, Microbial counts.

1. INTRODUCTION

The increase in human population has resulted to decrease in soil fertility due to continuous cropping in order to meet up with food needs of the growing population. Consequently, efforts have been made to improve soil fertility. These include application of farmyard manures, organic fertilizer and lime to the soil. Animal manures are commonly used over others due to its low cost and environmental friendliness. Animal manures when applied to the soil, adds nutrients to the soil, helps to stabilize soil aggregates, enhances microbial activities and soil structure etc. The importance of the use of animal manures offers many benefits such as safe environmental and sustainable soil fertility (UNEP, 2011).

The usefulness of animal manures depends on factors such as microbial diversity in the manure, the quality of organic manure, favorable physico-chemical properties such as the provision of sufficient aeration (Partanen et al., 2010; Chatterjee et al., 2013). Fungi, Bacteria and actinomycetes are the dominant microbial species in the decomposition of animal manures (Karnchanawong and Nissaikla, 2014). These microbial degradation activities are made possible by the activities of enzymes through hydrolysis of complex macromolecules present during metabolism of organic manures (Delgado et al., 2004). The amount and rate of bacteria, fungi and actinomycetes activities are determined by the level of readily metabolizable substrate found in the manure coupled with many factors such as temperature, soil pH and moisture level under aerobic conditions (Yu et al., 2007; Rebollido et al., 2008).

Dynamics of enzymatic activity in amended soils is commonly observed from thirty days to three years following application of soil amendments (Dick, 1994). Microorganisms and plant roots produced enzymes which catalyze nutrient cycles reaction (Green et al., 2007; Speir and Ross, 1978). Plant roots facilitate enzymes activity by creating favorable microhabitats such as water, increased porosity and a diversity of compounds for microorganisms (Dick, 1997). The enzymes that are critical in hydrolysis of both P and anhydrides of H_3PO_4 esters are termed phosphatases (Alexander, 1977). Phosphatases are distinctly different not only because of the dynamic nature of the substrates hydrolyzed but also by different pH ranges due to their optimal activity. Example are; acid phosphatase, optimal pH (4-6), neutral phosphatase, optimal pH (7) and alkaline phosphatase, optimal pH 8-10 (Speir and Ross, 1978). Acid phosphatase helps complete the phosphorus (P) cycle by catalyzing ester-phosphate hydrolysis producing PO_4^{3-}as an end product (Havlin et al., 2005).

The phosphates which are present in a soil are heterogeneous. In soils, the phosphatases are derived from the soil organisms with bacteria contributing more than the others (Anwesha et al., 2012). The changes in phosphatase activity in soils could be related to the number of biomolecules, inorganic and organic phosphatases (Chen et al., 2004). phosphatase activity in the soil system strongly depends on the soil moisture level and environmental temperature (Huang et al., 2011). There is a dearth of information on the effectiveness of animal manures at each stage of decomposition. Also, there is no specific information on the changes of phosphates activity and microbial population dynamics in soils as the manures are mineralized. Consequently, the study was carried out

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to address the paucity of information on changes in phosphates and microbial dynamics following organic amendment in the soil of the study area.

2. Materials and Methods

2.1 Study Area

The study was conducted at Domita farm in Uyo City, Nigeria. The study area is located between latitude 50^0 01'05" and 50^0 01'05" N and longitude 7^0 59' 50" and 7^0 59' 55". The soil belongs to the soil order ultisol. The soils of the study location are often referred to as the "Acid sand" in Southeastern Nigeria and were derived from coastal plain sands (Reyment, 1965). The area receives average annual rainfall in the range of 2500 mm to 3000 mm while mean daily temperature varies from 25 ^{0}C to 28 ^{0}C. Relative humidity is high throughout the year, varying from 75-80%. Two climatic seasons namely the rainy and dry seasons are prominent in the area. While the dry season starts from the month of November and ends in the month April, the rainy season commences in the month of May and terminates in the month of October (Peters et al., 1989; Usoro and Akpan, 2010). These climatic factors favour luxuriant tropical rainforest with multiple tree species, teaming population of insects and fauna of extremely high terrestrial and aquatic biomass. Farming, fishing and hunting are the major socio-economic activity in the area. *Dioscorea spp*, *Manihot spp*, *Elaeis guinensis* and *Zea mays* are widely cultivated in the area.

2.2 Field Study

The experimental field was cleared, ploughed, harrowed and layout mapped out. Cured poultry and swine manures obtained from University of Uyo Teaching and Research Farm were applied in their natural state (commonly used form by farmers) at the rate of 30 t ha^{-1} each. The treatments were laid out in a randomized complete block design replicated three times with each plot measured 3 m ×3 m giving a total of six plots. The treatments were allowed to decompose for a period of ninety days. Soil was sampled at 0-15 and 15-30 cm depths during the period of decomposition at day 0, 14, 28, 42, 56, 70 and 84. A total of eighty-four samples were taken for enzymes and microbial analysis using soil auger, sterilized polythene bags and stored in an ice-packed cooler. In addition, twenty-four samples were also obtained to determine physico-chemical properties of the soil before and after decomposition of manures. In preparation for soil laboratory analysis, soil samples were weighed, air-dried and allowed to passed through a 2-mm mesh sieve to obtain the fine earth fraction. The particle size analysis was determined using the hydrometer method (Gee and Or, 2002). Moisture content was determined gravimetrically using the equation below:

$$\theta ms = \frac{\text{Wet soil sample} - \text{dry soil sample}}{\text{Dry } soil\ sample} \times \frac{100}{1}$$

where $\theta ms =$ gravimetric moisture content(saturated), (%). The bulk density was by the core sampler method while Soil pH was measured in 1:2.5 soil-water suspension ratio using a standard pH meter (Grossman and Reinsch, 2002; Thomas, 1996). Soil organic carbon was determined by the Walkley and black wet oxidation procedure while total nitrogen was obtained by the Kjeldahl method (Nelson and Sommers, 1996; Bremner, 1996). Available phosphorus was determined using the method of Graetz and Nair (Graetz and Nair, 2000). Exchangeable bases were extracted using 1N NH_4AoC buffered to pH 7. After extraction, exchangeable calcium and magnesium were determined by the EDTA (Ethylene diaminetetracetic acid) titration method (Chapman, 1965). Exchangeable acidity was extracted using 1N KCl and then titrated with 0.05 N NaOH (Mclean, 1982). Effective cation exchange capacity (ECEC) was obtained as sum of the values of exchangeable bases and acidity. For manure analysis, the samples were air-dried, hammer milled to obtain particles that are less than 1.5 mm in size. The elemental content of the manure was extracted by digesting with HNO_3 and H_2SO_4 while other properties of the manure (pH, organic matter and organic carbon) were determined using standard procedures (Kaira and Maynard, 1991; Cater, 1993).

2.3 Microbial Analysis of Treated Soil

The microbiological analysis was determined by methods as outlined (Adesemoye et al., 2006; Oyeleke and Manga, 2008; Rahah et al., 2006). A gramme of soil sample obtained from treated soils was serially diluted in tenfold upto 10^6 tubes. Then l ml from the 10^6 tubes was aseptically inoculated into nutrient agar containing plates, glycerol agar and potato dextrose agar using a pour plate method. 100 ppm of nystatin and gentamycin growth inhibitors was added to nutrient and potato dextrose agar, respectively. All plates were allowed to set, inverted and incubated; nutrient agar-bacterial plates were incubated at for 24 hours at 37 ^{0}C temperature while potato dextrose agar plates (fungi, actinomycetes) were incubated for 72 hours at ambient laboratory temperature ($28\pm20\ ^0$C). After incubation period, total bacterial, fungal and actinomycetes colonies on plates were enumerated using colony counter B bran (England).

The colonies were multiplied by reciprocal of the dilution factor to obtain the plate count per gramme of soil sample and recorded as $Cfug^{-1}$. The colonies were repeatedly subculture onto fresh nutrient and potato dextrose agar media to obtain pure isolates for characterization using cultural, morphological and standard biochemical test as described (Cheesebrough, 2006). The biochemical testconducted include motility, catalase, methyl red rest, urease activity, coagulase test, citrate test, carbohydrate fermentation test, oxidase test and spore test. The fungal isolates were identified using the method of Oyeleke and Qkusanmi [39] which was based on the colour of aerial hyphae and substrate mycelium, conidial arrangement and arrangement of hyphae (Oyeleke and Qkusanmi, 2008). Actinomycetes isolates were characterized and identified as outlined by Eka and Fogathy (Eka and Fogathy, 1972).

2.4 Determination of Phosphomoesterase (Acid and Alkaline phosphatases).

A gramme of moist soil sample was obtained and added to a 50 ml Erlenmeyer flask (Hayes et al., 2007). 2 ml of toluene and 4 ml of Acetate buffer were added (pH 6.5 for assay of acid phosphatase or pH 11 for assay of alkaline phosphatase). Then l ml of P-nitrophenyl phosphate solution was added and the flask swirled for some seconds to ensure that the content was thoroughly mixed. The flask was stopped and was incubated for 1 hour at 37 ^{0}C temperature. After one hour the stopper was taken away and l ml of 0.5 m $Cacl_2$, and 4 ml of 0.5 m NaOH were added and the flask swirled for some seconds to mix the contents properly. The soil in the suspension was then filtered using Whatman no.42 filter paper. Colorimeter was used to obtain the value for phosphomonoesters at the wavelength of 420 nm, and the result recorded as Mg g^{-1} soil.

2.5 Statistical Analysis

The data obtained was subjected to analysis of variance (ANOVA) using the software package (SAS Institute, 1999). Means were separated using Duncan Multiple Range Test at 5% level of probability. The relationship between phosphates activity and microbial counts was evaluated using correlation analysis.

3. Result and Discussion

3.1 Manure Characterization

The chemical characteristics of the manures are presented in Table1. The Manure analysis results showed that organic matter, available phosphorus and pH were higher in poultry manure, indicating that poultry is of better quality compared to swine manure. It has been reported that animal manures differ greatly depending on type of the animal, diet fed to the animal, management system and age (Chadwick et al., 2000). However, on comparative basis, total nitrogen, organic carbon and C/N ratio were higher in swine manure than poultry manure. The higher C/N in swine manure indicates that microbial growth will be better in poultry manure since higher value of C/N ratio has been reported to reduce microbial growth by decreasing the mineralization of soil N (Azam, 2002). In addition, the findings suggest that mineralization would be faster in poultry manure than swine manure.

Table 1: Chemical Characteristics of the Manures used for the Study

Properties	Poultry manure (PM)	Swine manure (SM)
Total nitrogen g kg^{-1}	35.8	38.2
Organic matter g kg^{-1}	702.3	637
Organic carbon g kg^{-1}	348	390.8
Available phosphorus g kg^{-1}	17.2	12.0
C/N	9.7	10.2
pH(H_20)	6.62	6.60

The soil physico-chemical properties of the manure amended and control sites are shown in Table 2. Sand particle size ranged from 830.67 mg kg^{-1} to 871.33 mg kg^{-1} at the surface layer while subsurface ranged between 8945.33 mg kg^{-1} to 872.67 mg kg^{-1}. Silt ranged from 42.33 mg kg^{-1} to 69.33 mg kg^{-1} at the surface layer while subsurface ranged from 26.00 mg kg^{-1} to 52.00 mg kg^{-1}. Clay ranged from 83.33 mg kg^{-1} to 107.67 mg kg^{-1} at the surface layer while subsurface ranged from 83.33 mg kg^{-1} to 119.00 mg kg^{-1}. The dominance of sand particle size is a clear reflection of sandy nature of the coastal plain sands from which the soils were from as well as the influence of leached profiles under high annual rainfall conditions. Clays were higher than silt. The highest content of clay was observed at the subsurface layer of poultry manure treated soil after three months of manure decomposition. High deposition of clay observed at the subsurface layer may be due to clay migration by lessivage through the process of eluviation (Essoka and Esu, 2001).

Moisture content ranged from 19.33 % to 25.27 % at the surface layer while subsurface varied from 20.91 % to 26.87 %. Moisture content of both the manure amended and control sites increased significantly ($P<0.05$) with increased soil depth. This finding is in accord with the report of Aluko and Onyedele who observed that the use of animal manure is essential in improving water retention capacity of the soil (Aluko and Onyedele, 2005). Bulk density ranged from 1.30 Mg m^{-3} to 1.42 Mg m^{-3}at the surface layer while subsurface layer ranged from 1.38 Mg m^{-3} to 1.47 Mg m^{-3}. Bulk density increased significantly ($P<0.05$) with increased soil depth and the values were below the critical minimum values which suggests possible impairment of root growth. The soil pH ranged between 4.40 to 5.37 at the surface layer but varied from 4.07 to 4.97 at subsurface layer. The highest value of soil pH was recorded at the surface layer treated with poultry manure which could be due to higher pH value of poultry manure relatives to the swine manure. The soil pH decreased significantly ($P<0.05$) with increase in soil depth.

Generally, the pH varied from moderately acidic to extremely acidic in the amended and control soils. Organic carbon ranged between 1.31 gk g^{-1} to 2.20 gk g^{-1} at surface layer, but 1.08 gk g^{-1} to 2.59 gk g^{-1} at subsurface layer. Organic carbon of both treated and untreated soil decreased significantly ($P<0.05$) with increase in soil depth. Organic carbon was relatively higher in soil treated with swine manure as compared to poultry manure, which could be due to higher amount of carbon in the swine manure. Total nitrogen ranged between 0.06 gk g^{-1} to 0.11 gk g^{-1} at the surface layer, but subsurface ranged between 35.67 gk g^{-1} to 69.00 gk g^{-1}.The amount of total nitrogen was slightly higher in swine manure as compared to poultry manure, the value is a reflection of higher content of total nitrogen value in the swine manure. The available phosphorus decreased significantly ($P<0.05$) with increase in depth. The available phosphorus was not deficient in both treated and untreated soil considering 10-16 mg kg^{-1} being the critical level for crop production (Adeoye and Agboola, 1985).

Exchangeable acidity ranged between 1.85 Cmol kg^{-1} to 3.39 Cmol kg^{-1} at the surface soil while subsurface soil ranged from 1.96 Cmol kg^{-1} to 3.47 Cmol kg^{-1} The exchangeable acidity increased non-significantly with soil depth at both treated and untreated soil. Calcium ranged between 4.07 Cmol kg^{-1} to 10.13 Cmol kg^{-1} at the surface layer, but 4.03 Cmolkg^{-1} to 7.57 Cmolkg^{-1} at subsurface layer. The calcium content of both treated soil and untreated were higher considered the critical value of 5.0 Cmol kg^{-1} (Amalu, 1997). Calcium dominated other cations thus the soils are expected to have better soil aggregate stability. The higher the calcium contents of the soil, the more supportive aggregate stability of the soil becomes since calcium acts as a binding agent aggregating soil particles (Baver et al., 1978). Calcium content decreased significantly ($P<0.05$) with increase in soil depth. Magnesium ranged between1.80 Cmol kg^{-1} to 3.99 Cmol kg^{-1} at the surface layer, but subsurface ranged between 1.63 Cmol kg^{-1} to 2.82 Cmol kg^{-1}. Magnesium were high based on critical value of 0.50 Cmol kg^{-1} (Onyekwere et al., 2003).

Sodium ranged between 0.06 Cmol kg^{-1} to 0.07 Cmol kg^{-1} at the surface soil, but subsurface ranged between 0.08 Cmol kg^{-1} to 0.20 Cmol kg^{-1} at the surface layer but subsurface layer ranged between 0.05 Cmol kg^{-1} to 0.06 Cmol kg^{-1}. Potassium ranged between 0.08 Cmol kg^{-1} to 0.20 Cmol kg^{-1} at the surface layer but subsurface layer ranged between 0.08 Cmol kg^{-1}. The K and Mg content of the soils were adequate considering the 0.16-0.25 Cmol kg^{-1} and 0.2-0.4 Cmol kg^{-1} critical levels respectively reported (Adeoye and Agboola, 1985). Effective cation exchange capacity ranged between 8.26 Cmol kg^{-1}to17.23 Cmol kg^{-1} at surface soil while in the subsurface layer, it ranged from 7.84 Cmol kg^{-1} to 13.93 Cmol kg^{-1}. The ECEC decreased significantly ($P<0.05$) with increase in soil depth. and may be due to decreasing organic matter with depth since organic matter level has been reported to contribute significantly to ECEC values (Baver et al., 1978). Percentage base saturation ranged between 72.73 % to 82.05 % to 76.24 % at the surface layer but subsurface layer ranged between 73.83 % to 76.24 %. The percentage base saturation of the soil was adequate considering the 50 % critical level reported (Landon, 1984).

Table 2: Physico-chemical properties of the soil of the study area at 0-15 cm and 15-30 cm soil depth tested parameters

	0-15P_1	**15-30P_1**	**0-15S_1**	**15-30S_2**	**0-15P_2**	**15-30P_2**	**0-15S_2**	**15-30S_2**
Sand ($mgkg^{-1}$)	854.00b	85.200b	845.33a	848.33a	871.33a	872.67a	830.67b	854.00a
Silt ($mgkg^{-1}$)	42.33acd	26.00d	69.33a	32.67cd	45.33abc	44.00abc	61.67ab	52.00abc
Clay ($mgkg^{-1}$)	103.67abc	122.00a	85.33c	119.00a	83.33c	83.33c	107.67ab	94.00bc
MC (%)	25.27b	25.27b	25.86ab	26.87a	19.33d	21.85c	19.93dc	20.91cd
BD (mgm^{-3})	1.30cd	1.38bc	1.27c	1.40b	1.41b	1.47ab	1.42ab	1.45a
pH (H_2O)	5.37a	4.97b	4.73ab	4.47bc	4.40c	4.07d	4.40c	4.30cb
OC (gkg^{-1})	2.20ab	2.03c	2.59a	2.27a	1.31c	1.08ab	1.53bc	1.25c
TN (gkg^{-1})	0.11a	0.09bc	0.12a	0.10b	0.06de	0.04e	0.10b	0.05de
AVP ($mgkg^{-1}$)	69.67a	45.67bc	65.00a	69.00b	45.00bc	39.00cd	43.33bcd	35.67d
EA ($Cmolkg^{-1}$)	3.28a	3.43a	3.39a	3.47a	1.85b	1.96b	2.05b	2.25b
Ca ($Cmolkg^{-1}$)	10.13a	7.53b	9.17a	7.57b	6.37b	5.67c	4.07c	4.03c
Mg ($Cmolkg^{-1}$)	3.44a	2.82c	3.99a	2.63b	1.87d	2.37cd	1.80c	1.63c
Na $Cmolkg^{-1}$	0.07a	0.06a	0.06ab	0.07a	0.06ab	0.05bc	0.06ab	0.05c
K $Cmolkg^{-1}$	0.16bc	0.17c	0.20a	0.19ab	0.14c	0.10d	0.08c	0.08c
ECEC $Cmolkg^{-1}$	17.23a	13.87b	16.80a	13.93b	10.29c	10.15c	8.26c	7.84c
BS (%)	80.06a	76.24b	79.95a	75.05cb	82.02a	80.69a	72.73c	73.83cb

Key: Mean values with the same letter (s) within the rows are not significantly different from one another at $P<0.05$. MC - moisture content, BD – bulk density, OC –organic carbon, TN – total nitrogen, AVP – available phosphorus, EA – exchangeable acidity, Ca – calcium, Mg – Magnesium, Na – Sodium, K – Potassium, ECEC – effective cation exchange capacity, BS – base saturation, P_1 and S_1-represent soil treated with poultry and swine manures respectively, P_2 and S_2 – represented soil untreated with poultry and swine manures respectively.

Alkaline phosphatase ranged between 0.38 Mg g^{-1} to 4.94 Mg g^{-1} in soil amended with poultry manure (Table 3) while in soil amended with swine manures it varied from 0.68 Mg g^{-1} to 4.80 Mg g^{-1}. Acid phosphatase ranged between 0.42 Mg g^{-1} to 3.02 Mg g^{-1} in soil amended with poultry manure but in soil treated with swine manure its value ranged between 0.11 Mg g^{-1} to 2.38 Mg g^{-1}. The optimal activity of alkaline and acid phosphatase were observed at second week of decomposition on the surface layers of the soil and dropped toward the end of decomposition. This study revealed that the decomposition of animal manures strongly influenced soil enzymatic activity. Because of the high level of manures at the early stage of decomposition, there was significant increase in enzymes released at week 2 and probably may be due to sufficient amount of substrate for greater microbial density and higher enzymes production (Yuan and Yue, 2012). It could also be due to higher microbial species at early stage of decomposition. This study corroborated with the findings reported that fungi and bacteria are capable of producing phosphatase in the soil system (Tarafdar and Chhonkar, 1979).

Table 3: Changes in alkaline and acid phosphatase activity in top-soil treated with poultry and swine manure.

Poultry manure treated soil				
Duration	**Alkaline phosphatase** Mg g^{-1}		**Acid Phosphatase** Mg g^{-1}	
	0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**
0 Week	1.74b	0.38a	0.55a	0.42a
Week 2	4.94a	2.55cb	3.02a	1.13b
Week 4	3.05a	2.68b	1.52b	2.67a
Week 6	2.16cb	2.51b	1.42a	1.84a
Week 8	2.10cb	2.55b	1.36ab	2.05a
Week 10	1.97b	3.50a	0.56b	1.84a
Week 12	1.91a	2.53ab	0.60b	1.91a
Swine manure treated soil				
0 Week	1.21a	0.68b	0.47b	0.11b
Week 2	4.80a	1.89c	2.38a	1.51a
Week 4	2.18b	4.52a	1.58b	2.15ab
Week 6	2.22b	4.57a	1.45a	2.34a
Week 8	1.80b	3.31b	1.03b	2.23a
Week 10	1.55c	2.76a	0.82b	2.18a
Week 12	1.35b	3.07a	0.53b	2.09a

Key: means values with the same letter(s) within the rows are not significantly different from one another at P<0.05.

Total heterotrophic bacteria densities ranged between 1.27×10^4 Cfu g^{-1} to 8.63×10^7 Cfu g^{-1} in treated soil with poultry manure (Table 4) whereas in soil amended with swine manure, total heterotrophic bacteria also ranged between 1.40×10^4 Cfu g^{-1} to 8.0×10^7 Cfu g^{-1}. Total fungal counts ranged between 1.63×10^3 Cfu g^{-1} to 4.67×10^6 Cfu g^{-1} in soil amended with poultry manure while in soil treated with swine manure fungal counts 2.00×10^3 Cfu g^{-1} to 5.67×10^6 Cfu g^{-1}. Total actinomycete counts ranged between 1.10×10^3 Cfu g^{-1} to 6.60×10^5 Cfu g^{-1} in amended soil with poultry manure Comparatively, in soil amended with swine manure, total actinomycetes

counts ranged between 1.40×10^3 Cfu g^{-1} to 5.30×10^5 Cfu g^{-1} . The results showed significant differences in the microbial counts during the twelve weeks period of decomposition. There was an initial increase in the microbial counts at the surface layer week 2. This finding is in consonance with the finding investigated mineralization and changes in bacterial population dynamics in soils following organic manure amendment and noted an initial rise in microbial density in the organic amendment treated soils which could be due to the use of the animal manure as source of energy by soil bacteria, fungi and actinomycetes (Kunc et al., 1985).

The results from this study revealed a decrease in the microbial counts with increase in soil depth at 0 week and week 2. At week 4 to week 12, the subsurface layer recorded highest microbial counts with the peak at week 6. This may be attributed to humid rainfall characteristics of the area that favours the movement of manures down to the subsurface layer. The counts revealed total heterotrophic bacteria were the dominant soil organisms followed by fungi and then actinomycetes.

The dominance of the heterotrophic bacteria could be due to the ability of the soil organism to tolerate wide variations of the soil properties (Brady, 1984). Similarly, the high fungi count was attributed to the low pH of the soils in the study area since low pH favours fungi diversity (Landeoker, 1990). The low count of actinomycetes observed could be attributed to the acidic nature of the studied soils which does not encourage high proliferation of actinomecetes (Brady, 1984). Comparatively, soil treated with swine manure recorded highest microbial counts than poultry dropping treated soil.

Table 4: Total microbial counts (Cfu g^{-1}) in top-soil and sub-soil treated with poultry and swine manures

Microbial counts	**Soil depth (cm)**	**Week 0** ($\times10^3$ $\times10^4$ Cfu g^{-1})	**Week 2** ($\times10^4$ $\times10^6$Cfu g^{-1})	**Week 4** ($\times10^4$ $\times10^5$ $\times10^6$Cfu g^{-1})	**Week 6** ($\times10^5$ $\times10^6$ $\times10^7$Cfu g^{-1})	**Week 8** ($\times10^4$ $\times10^5$ Cfu g^{-1})	**Week 10** ($\times10^3$ $\times10^4$ $\times10^5$Cfu g^{-1})	**Week 12** ($\times10^3$ $\times10^4$ $\times10^5$Cfu g^{-1})
THBC (pd)	0-15	1.80×10^4a	1.67×10^6a	5.90×10^6c	5.40×10^7b	1.40×10^5a	6.47×10^4c	4.53×10^4c
	15-30	1.27×10^4a	1.27×10^6b	8.13×10^6ab	8.63×10^7a	1.57×10^5b	6.83×10^4c	5.37×10^4c
THBC (sm)	0-15	2.64×10^4a	1.97×10^6a	7.67×10^6b	7.00×10^7ab	2.29×10^5ab	1.57×10^5a	1.43×10^5a
	15-30	1.40×10^4a	1.77×10^6a	9.03×10^6a	8.10×10^7a	3.40×10^5a	1.73×10^5a	1.60×10^5a
TFC (pd)	0-15	2.10×10^3a	5.33×10^4a	3.40×10^5a	3.33×10^6b	5.67×10^4a	2.43×10^4c	2.33×10^3b
	15-30	1.63×10^3b	4.00×10^4a	4.33×10^5a	4.67×10^6b	4.67×10^4a	2.93×10^4cb	3.00×10^3ab
TFC (sm)	0-15	5.47×10^3c	5.30×10^4a	6.33×10^5a	3.33×10^6b	7.33×10^4a	4.00×10^4a	2.33×10^3b
	15-30	2.00×10^3a	4.22×10^4a	5.67×10^5a	5.67×10^6a	6.33×10^4a	3.67×10^4ab	3.67×10^3a
TAC (pd)	0-15	1.60×10^3b	1.50×10^4ab	4.80×10^4c	3.83×10^5b	1.10×10^4a	3.03×10^4a	1.80×10^3b
	15-30	1.10×10^3a	1.17×10^4b	5.47×10^4cb	6.60×10^5a	1.10×10^4a	4.33×10^4a	2.93×10^3b
TAC (sm)	0-15	1.93×10^3a	1.67×10^4a	6.07×10^4ab	5.30×10^5a	1.80×10^4a	4.57×10^3a	2.20×10^3c
	15-30	1.40×10^3a	1.47×10^4ab	6.90×10^4a	6.33×10^4a	2.47×10^4a	4.90×10^3a	3.40×10^3a

3.2 Diverse Microbial Species Isolated from the Test Soil.

Based on the results as shown in (Table 5), the bacterial isolates were identified as *pseudomonas dinimuta,micrococcus varians,B*acillus *subtilis*, *Escherichia* coli, *Streptococcus pyrogenes*, *Bacillus cereus* and *staph aureus* Actinomucete isolates were *Actinoborocallus sp*, *Streptomycesn sp* and *Micromonospora* sp. Fungal isolates were *Aspergillus flavus*, *Aspergillus niger, Aspergillus fumigatus, Aspergillus paraiticum*, *Rhizopus Stolonifer*, *Mucor mucedo*, *Fusarium Oxytoca*, *Fusarium* t*rincinctum*, *Fusarium semitectum*, *Humicola fuscoatra*, *Penicillium expansum*, *Penicillium frequentans*, *Penicillium* n*otatum*, *Paecillomycessp*, *Verticillium* a*lboatrum*, *Botrytissp*, *Cladosporium fulvum*, *monilia stilophila*, *Aureobasidium pullulans* etc.

Table 5: Microbial isolates from the test soil

S/N	**BACTERIA**	**FUNGI**	**ACTINOMYCETES**
1.	*Micrococcus varians*	*Aspergillus flavus*	*Actinoborocallus sp*
2.	*Pseudomonas diminuta*	*Aspergillus niger*	*Streptomyces sp*

3.	*Streptococcus Pyrogenes*	*Aspergillus fumigates*	*Micromonospora sp*
4.	*Baccilus cereus*	*Aspergillus parasiticus*	
5.	*Arthrobacter sp*	*Rhizopus stolonifer*	
6.	*Escherichia coli*	*Mucor mucedo*	
7.	*Pseudomonas aeruginosa*	*Fusarium oxytoca*	
8.	*Baccilus subtilis*	*Fusarium trincinetum*	
9.	*Staph aureus*	*Fusarium semitectum*	
10.		*Humicola fuscoatra*	
11.		*Penicillium expansum*	
12.		*Penicillium freqentans*	
13.		*Penicillium notatum*	
14.		*Paecillomyces sp*	
15.		*Verticillum alboatrum*	
16.		*Botrytis sp*	
17.		*Cladosporium fulvum*	
18.		Monilia stilophila	
19.		Aureobasidium pullulans	

3.3 Relationship between Microbial Densities and Alkaline and Acid phosphatase

The relationship between microbial densities and alkaline and acid phosphatase are shown in (Table 6). Bacterial density showed a positive and significant correlation with alkaline phosphatase at the surface layer and subsurface layer (r=0.766*; 0.538*) in soil treated with poultry respectively. This positive and significant relationship characterized that bacteria are responsible for the production of alkaline phosphatase as reported (Tarafdar and Chhonkar, 1979).There was a positive and non-significant correlation between bacteria density and acid phosphatase at the surface layer (r=0.203), but the relationship between bacterial density and acid phosphatase at subsurface layer was non-significant and negatively correlated (r=-0.399). Actinomycete density showed a positive and non-significant relationship with alkaline phosphatase at the surface and subsurface layer (r=0.438; 0.219) respectively in soil treated with poultry manure.

Actinomycete density and acid phosphatase was also positive and non-significant at both surface and subsurface layer treated with poultry manure (r=0.389; 0.154) respectively. This relationship implies that increase in actinomycete counts will also increase alkaline and acid phosphatase in the soil system. Fungal density and alkaline phosphatase was positively and non-significantly correlated at the surface layer amended with poultry manure (r=0.306), while subsurface layer was negatively and non-significantly correlated (r=-0.197). Fungal density and acid phosphatase was positively and significantly correlated at the surface soil treated with poultry manure (r=0.656*), Suggesting that both are from the same origin while the subsurface layer relationship was positive and non-significant (r=0.265).

In soil treated with swine manure, bacterial density showed a positive and significant relationship with alkaline phosphatase at the surface layer (r=0.675*), implying that both are from the same origin. The relationship between bacterial count and alkaline phosphatase at the subsurface layer was positive and non-significant (r=0.454). Bacterial density and acid phosphatase was positively and significant correlated at the surface layer (r=0.620*), but the subsurface layer was positive and non-significant (r=0.049). Actinomycete density and alkaline phosphatase was positive and significantly correlated at the sub-soil treated with swine manure (r=0.635*) while surface layer was positive and non-significant (r=0.396).

Actnomycete density and acid phosphatase was positive and non-significantly correlated at surface and subsurface layer (r=0.463; 0.408) respectively. This relationship implies that increase in actinomycete counts will increase alkaline and acid phosphatase in the soil when treated with swine manure. Fungal density and alkaline phosphatase was positive and non-significant correlation at both surface and subsurface layer treated with swine manure (r=0.463; 0.408) respectively. Fungal density and acid phosphatase was positive and significantly correlated at the surface layer treated with swine manure (r=0.607*) while subsurface layer was positive and non-significantly correlated (r=0.487). This relationship implies that increase in fungal counts will increase acid phosphatase activity in soil.

Table 6: Pearson Correlation between Microbial Densities and Phosphatase Activities in Top-soil and Sub-soil Treated with Poultry and Swine Manures (n=5).

Statistical pairs	**r - values**	
Poultry manure treated soil	**Top-soil**	**Sub-soil**
Bacterial density and alkaline phosphatase	0.766*	0.538*
Bacterial density and acid phosphatase	0.203^{ns}	-0.399^{ns}
Actinomycete density and alkaline phosphatase	0.438^{ns}	0.219^{ns}
Actinomycete density and acid phosphatase	0.389^{ns}	0.154^{ns}
Fungal density and alkaline phosphatase	0.306^{ns}	-0.197^{ns}
Fungal density and acid phosphatase	0.656*	0.265^{ns}
Swine manure treated soil		
Bacterial density and alkaline phosphatase	0.675*	0.454^{ns}
Bacterial density and acid phosphatase	0.620*	0.049^{ns}
Actinomycete density and alkaline phosphatase	0.396^{ns}	0.635*
Actinomycete density and acid phosphatase	0.478^{ns}	0.363^{ns}
Fungal density and alkaline phosphatase	0.463^{ns}	0.408^{ns}
Fungal density and acid phosphatase	0.607*	0.487^{ns}

Key: *-significant at 5% probability level.

4. Conclusion

The findings of this study showed that addition of animal manures significantly improved the soil properties, phosphatase activities and microbial population dynamics. Bacterial density showed significant positive relationship with alkaline phosphatase at both the surface and subsurface layers. This accounts to an increase in fertility status of the test soil as reflected in the study. Hence, the use of animal manure would be a promising and practical approach to sustaining soil productivity. Further studies on the dynamics of phosphatase enzyme under the influence of animal manures in relation to phosphorus availability should be carried out.

References

Adeoye, G.O., Agboola A.A., 1985.Critical Levels of Soil pH, available P, K, Zn and Mn and Maize ear leaf content of P, Cu and Mn in Sedimentary Soil of Southwest Nigeria. Fertilizer Research, 6, 65-71.

Adesemoye, A.O., Opere, B.O., Makinde, S.C.O., 2006. Microbial Content of Abattoir Waste Water and its Contaminated Soil in Lagos, Nigeria. African Journal of Biotechnology, 5 (20), 1963-1968.

Alexander, M. 1977. Introduction to Soil Microbiology. 2nd Edu., John Wiley and Sons Inc., New York, ISBN-13: 9780894645129, page 467.

Aluko, O.P. Onyedele, D.J., 2005. Influence of Organic Waste Incorporation and Changes in Selected Soil physical Properties. Journal of Applied Science, 5(2), 357-362.

Amalu, U.C., 1997. Evaluation of Properties of Selected Soils of Cross River State Area and their Management for Increase Cassava Yields. Global Journal of Pure and Applied Science, 4 (3), 243-249.

Anwesha, B., Sanghamitra, S., Sribir, S., 2012. Soil Phosphatase Activity of Agricultural Land: A Possible Index of Soil Fertility. Agricultural Science

Research Journal, 2 (7), 412-419.

Aune, J.P., Lal, R., 1997. Agricultural Product Productivity in the Tropics and Critical Limits of Oxisol, Ultisol. Journal of Tropical Agriculture,74, 96-103.

Azam, F., 2002. Added nitrogen interaction in the soil-plant system – A review. Pakistan Journal of agriculture, 1, 54–59.

Baver, I.D., Gardner, W.H., Gardner, W.R., 1978. Soil Physics. Fourth Edition., John and Son's. New York.

Brady, N.C., 1984. The Nature and Properties of Soils. New York: Macmillan Publishing Company, pp.10-593.

Bremner, J.M., 1996. Nitrogen Total Sparks, D.L, (ed). Methods of Soil Analysis, Part 3, Chemical Method 2nd Ed. SSA Book Series No.5, SSSA, Madison, w1. Pp 1685-1121.

Cater, M.R., 1993. Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Ontario, Canada.

Chadwick, D.R.., John, F.,.Pain, B., Chambers, B.J., Williams, J.C., 2000. Plant uptake of nitrogen from the organic nitrogen fraction of animal manures: a laboratory experiment. Journal of Agricultural Science. Camb., 134, 159–168.Chapman, H.D., 1965. Method of Soil Analysis Part2. Chemical and Microbial Properties. (A Black Series (eds).

Chatterjee, N., Flurry, M.., Hinman, C., Cogger, C.G., 2013. Chemical and Physical Characteristics of Compost Leachates-A Review. Washington State University, Puyallup.

Cheesebrough, M. 2006. District Laboratory Practice in Tropical Countries. Part 2. Low Price Edition. Cambridge University Press, London, pp. 28-90.

Chen, C.R., Condron, L.M., Davis, M.R.., Sherlock R.R., 2004. Effect of Plant Species on Microbial Biomass Phosphorus and Phosphatase Activity in a Range of Grassland Soils. Biology and fertility of soil, 40, 313-322.

Delgado, A., Soleradel Rio, R.., Sales, D., Gareiamorales, J.L., 2004. Study of the Co-composting of Municipal Solid Waste and Sewage Sludge: Stability and Maturity. In: Bernal M, Moral R, Clement R, Paredes C (eds) Proceedings of the 11th Conference of the FAO on Recycling of Agricultural Municipal and Industrial Residues in Agriculture, Ramiran, Murcia, pp. 257-260.

Dick, R.P., 1994. Soil Enzyme Activities as Indicators of Soil Quality. In Doran, J. W. Coleman, D. C., Bezdicek, D. F., Stewart, B. A. (eds). Defining Soil Quality for a Sustainable Environment. Madison SSSA, pp. 107-124.

Dick, R.P., 1997. Soil Enzyme Activities as Integrative Indicators of Soil Health. In: Pankhust, C. E., Doube, B. M., Gupta, V. V. S. R (eds). Biological Indicators *of* Soil health, New York: CAB International. Pp. 121-156.

Eka, O.U., Fogathy, N.M., 1972. Descriptive Studies on a Streptomyces Species Part 1. Description and some Properties of the Microorganisms. West African Journal of Biological and Applied Chemistry, pp. 11-17.

Essoka, A.N., Esu, I.E., 2001. Physical Properties of Inland Valley Soil of Central Cross River State, Nigeria. 27th Annual Conference of Soul Science Society of Nigeria, Calabar, pp. 119-123.

Gee, G.W., Or, D., 2002. Particle size Analysis. In: Method of Soil Analysis Part 4 Physical *Method*. Soil Science Society of America, pp. 255-293.

Graetz, D.A., Nair, V.D., 2000. Phosphorus Sorption Isotherm Determination in: Methods of Phosphorus Analysis for soils, Sediments, Residual and Waters. Prierzynaki, G. M. (ed). Bull Bo.369, Southern Extension/Research Activity Information Exchange Groups, Kansas State University, Manhattan, Ks, 35-38.

Green, V.S., Stott, J.C., Cruz, D.E., Curi, N., 2007. Tillage Impacts on Soil Biological Activity and Aggregation in a Brazilian Cerrado Oxisol-soil and Tillage Research, 92, 114-121.

Grossman, R.B., Reinsch, T.G., 2002. Book Density and Linera Extensibility in: Dene, J. H., Toop, G.C. (eds). Methods of Soil Analysis, Part 3: Physical Methods: Soil Science America, Book No.5, ASA and SSA Madison, W1, pp. 201-228.

Havlin, J.L., Beston, J.D., Tisdale, S.L., Nelson, W.L., 2005. Soil Fertility and Fertilizers: An introduction to Nutrient Management Practice Hall, Upper Saddle River N. J. ISBN 0-13-626806-4.

Hayes, J.E., Richardson, A.E., Simpson, R.J., 2007. Components of Organic Phosphorus in Soil Extracts that are Hydrolyzed by Phatase and Acid Phosphatase. Journal of Soil Biology and Fertility, 32, 279-286.

Huang, W., Liu, J., Zhou, G., Zhang, D., Deng, Q., 2011. Effect of Precipitation on Soil Acid Phosphatase Activity in three Successional Forests in Southern China. Biogeosci., 8, 1901-1910.

Kaira, Y.P., Maynard, D.G., 1991. Method manual for forest soil and plant analysis. For Can., Northwest Reg., North. For. Cent., Edmonton, Alberta. Inf. Rep. NOR-X-319.

Karnchanawong, S., Nissaikla, S., 2014. Effects of Microbial Inoculation on Composting of Household Organic Waste using Passive Aeration Bin. Int., Recycle Org. Waste Agric., 3 (4), 113-119.

Kunc, F., Tichy, P., Vancura, V., 1985. 2-4 Dichlorophitexoxy Acetic Acid in the Soil: Mineralization and Changes in the Counts of Bacteria Decomposer. Versailles Ed. INRA Pbl (les Colloqes de I'NRA No.31).

Landeoker, M.E., 1990. Fundamentals of the Fungi. 3rd Edition, New Jersey: Prentice Hall Inc.

Landon, J.R., 1984. Booker Tropical Manure a Handbook for Soil Survey and Agricultural Land Evaluation in the Tropics and Sub Tropics. Booker International Limited. United Kingdom. Pp. 1961.

Mclean, E.O., 1982. Aluminium in: Method of Analysis. A black (ed) Agronomy No 9, Part 2 American Society of Agronomy, Madison W1, pp. 978-998.

Nelson, D.W., Sommers, L.E., 1996. Total Carbon, Organic Carbon and Organic Matter in*:* Methods of Soil Analysis part 3 Chemical Methods Sparks D. L. (ed). American Society of Agronomy/Soil Science Society of America. Madison, W1, pp. 091-1010.

Onyekwere, I.N., Ano, A.O., Ezenwa, M.I.S., Asunde, A.O., Bala, A., 2003. Assessment of Exchangeable Acidity Status and Management of Wetland Soil of Cross River State. Proceeding of 28th Annual Conference of Soil Science Society of Nigeria. (eds) pp. 52-59.

Oyeleke, S.B., Manga, S.B., 2008. Essential of Laboratory Practical in Microbiology. First Edition, Tobest Publishers, Minna, Nigeria. pp. 36-67

Oyeleke, S.B., Qkusanmi, T.A., 2008. Isolation and Characterization of cellulose Hydrolyzing Microorganisms from the Rumen of Ruminants. African Journal of Biotechnology, 7(9), 30.

Partanen, P., Hultman, L., Paulin, P., Auvinen, M., Romantschuk, M., 2010. Bacterial Diversity at Different Phases of the Composting Process. BMC Microbial 10:1-//. https://doi-org/10.1186/1471-2180-10-94 cross ref.

Peters, S.W., Udo, E.J., Obot, U.W., Okpon, S.W., 1989. Akwa Ibom State Physical Background Soil and Land Use and Ecological Problems. Technical Report of the Task Force on Soils and Land Use. Govt Printer. Uyo 602.

Rahah, A.B., Ijah, U.J., Manga, S.B., Ibrahim, M.L., 2006. Assessment of Physiochemical and Microbiological Qualities of Abattoir Waste Water in Sokoto, Nigeria. Nigerian Journal of Basic and Applied Science, 16(2), 145-150.

Rebollido, R., Martinez, J., Aguilera, Y., Melchork, R., Koerner, R., Stegmann, R., 2008. Microbial Populations during Composting Process of Organic Fraction of Municipal Solid Waste. Appl. Ecol. Environ. Res., 6(3), 61-67.

Reyment, R.A., 1965. Aspects of the Geology of Nigeria: The Stratigraphy of the Cretaceous and Cenosolo Deposits. Ibadan University Press, Pp.115.

SAS Institute. 1999. The SAS System for Windows. Release 8.0. SAS Institute, Cary, NC.

Speir, T.W., Ross, D.J., 1978. Soil Phosphatase and Sulphatase. In: Soil Enzymes, Burns, R. G. (Ed). Academic Press, London, pp. 197-250.

Tarafdar, J.C., Chhonkar, P.K., 1979. Phosphatase Production of Microorganism Isolated from diverse types of Soils. Zentralbi Bakteriol Naturwiss, 134(2), 19-24.

Thomas, G.W., 1996. Soil pH and Acidity in: Method of Soil Analysis Part 3. Chemical Methods SSA Book Series No.2 SSSA and ASA Madison WI, pp. 475-490.

United Nations Environment Program (UNEP). 2011. Towards a Green Economy: Pathways to Sustainable Development and Poverty Eradication, Nairobi.www.unep.org/green economy.

Usoro, E.J., Akpan, P.A., 2010. Akwa Ibom State Geographical Perspective Immediate Publication Limited Enugu. pp.23-51.

Yu, H., Zeng, G., Huang, H., Xi, X., Wang, R., Huang, D., Huang, G., Li, J., 2007. Microbial Community Succession and Lignocellulose Degradation during Agricultural Waste Composting. Biodegradaion, 18 (6), 793-802. https://doi.org/10.1007/s/0532-007-9108-8.

Yuan, B., Yue, D. 2012. Soil Microbial and enzymatic Activities across a Chronosequence of Chinese Pine Plantation Development on the loess Plateau of China. Pedosphere, (22), 1-12.