



Evaluating the Long-Term Effect of Integrated Nutrients of Pome, Cow Dung and NPK on Soil Chemical Properties under a Garden-Egg Field

Ipinmoye I. K.^a and Dayo-Olagbende O. G.^{b*}

^a Teaching and Research Farm, Faculty of Agricultural Sciences, Ekiti State University, Ekiti State, Nigeria.

^b Department of Agricultural Sciences, College of Sciences, Afe Babalola University Ado-Ekiti, Ekiti State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Soil amendments have been found to have a long-term effect on soil either positively or negatively, several soil management researches have been able to establish that sole application of inorganic fertilizer particularly nitrogen fertilizers can leave residual hydrogen ions in the soil which causes soil acidity over time. This study investigated the residual effects of different soil amendments of Palm Oil Mill Effluents (POME), cow dung, and NPK fertilizer, on soil chemical properties in two locations: Ekiti State University Teaching and Research Farm (EKSU) and Onu-ljelu High School Agricultural Farm. The aim is to evaluate the long-term impact of these commonly used organic and inorganic nutrient sources on soil properties. The research utilizes a randomized complete block design replicated three times to test the residual effect of the treatments. The research was divided

*Corresponding author: Email: dayoolagbendeog@abuad.edu.ng;

into first and second trial, the result presented here is based on the outcome of the second trial. At the termination of the residual trial, it was found that organic matter contents of soils treated with cow-dung and POME increased while that of the NPK fertilizer reduced. There was a decrease in soil pH on soils treated with NPK fertilizer but found to increase on soils treated with POME and cow-dung. Other nutrient elements including exchangeable cations were also found to increase in soils with POME and Cow-dung. This phenomenon was attributed to mineralization process which made nutrients stored-up in these organic materials to be released slowly and at the same time reducing their loss from the soil. It was concluded that nutrients from organic sources have a longer residual advantage to soil and can be used in combination with inorganic fertilizer for better soil management. The findings from these result provides valuable insights into the long-term effect of these soil amendments and can help farmers and researchers make informed decisions about their appropriate and sustainable use in agriculture.

Keywords: Cow-dung; POME; Soil; Long-Term; Residual.

1. INTRODUCTION

Agricultural productivity strongly depends on the health and fertility of the soil. Low soil fertility is one of the greatest biophysical constraints to production of agroforestry crops across the world [1]. Over the years, several agricultural practices and inputs have been used to boost the fertility of the soil as well as productivity, one of such practices is the application of organic materials like poultry manure [2] and inorganic fertilizers to soil to amend soil chemical properties and nutrient content [3,4]. Some researchers have also looked into the effect of split nitrogen fertilizer on soil properties [5] while others has established that integrated nutrient management can improve rice performance [6]. Palm oil mill effluent (POME) is a byproduct of the palm oil industry and has been considered a source of organic fertilizer due to its organic matter content. Palm oil mill effluent (POME) is a wastewater generated from palm oil milling activities which requires effective treatment before discharge into watercourses due to its highly polluting properties. This wastewater is a major issue in the production of palm oil as it is discharged directly into the soil where it literally destroys the soil physical and chemical properties. Pollution of soil and water caused by wastewater is one of the major global threats that our environment is facing today. One of the wastewater source is palm oil mill effluent (POME) final discharge. In palm oil producing countries like Malaysia and Nigeria, a large amount of POME is generate annually [7] It is estimated that for every tonne of crude palm oil produced, about 2.5 to 3.5 tonne of POME is generated [8]. POME contains biochemical oxygen demand (BOD) as well as chemical oxygen demand (COD) causing a reduction of the biodiversity and ability of aquatic ecosystem,

this is why it is considered the main source of water pollution in Malaysia [9]. Furthermore, if released into the water bodies particularly the river, it cannot be undone easily. This is because POME is generated in huge amounts at a time, it is very difficult to manage, and the treatment of this wastewater is expensive. Consequently, the cheapest and easiest way for this wastewater disposal that have been practiced in Malaysia is by discharging the treated POME to the nearby river or stream [8]. Characterization of POME had been conducted in various studies which only involve parameters that were listed as discharge standards by local environmental authorities and those that were significant to the results of the chosen treatment methods. It is comprised of different suspended materials which makes it one hundred times more polluted than the municipal sewage which has a high biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The effluent also contains higher concentration of organic nitrogen, phosphorus and different supplement substance making it a good source of nutrients for plant growth and development. Cow-dung on the other hand has been used widely in regions as a traditional organic fertilizer. Inorganic fertilizers particularly NPK is commonly used as a source of nutrient for plant development. In most parts of the world where cow is reared, about 9–15 kg dung/day is generated [10] and most of these wastes are generally meant for discarding because it may act as a source of pollution [11]. Cow dung is a very good source for maintaining the production capacity of soil and enhances the microbial population. But due to increasing population pressure and demand of food resources, there is a need of introducing a chemical fertilizer, pesticides and insecticides to the soil, which are disturbing the soil physiochemical properties including soil texture,

porosity, and water holding capacity and also disturbed the soil microbial population. While the effects of these nutrient sources on crop growth and yield have been well-documented, their residual effect on soil chemical property has received less attention. Understanding the long-term effect of these amendments on soil is crucial for sustainable agricultural practices. Therefore, this research study aims to evaluate the residual effect of POME, cow-dung and NPK fertilizer on soil chemical properties. By analyzing the changes in soil pH, organic matter content, nutrient availability and other key soil parameters.

2. METHODOLOGY

2.1 The Study Area

The experiment was conducted simultaneously at two distinct locations: the Ekiti State University Teaching and Research Farm (EKSU) and the Onu-Ijelu High School Agricultural Farm. Both sites are situated at an altitude of 332 meters above sea level, located in different forest regions of Ekiti state, Nigeria. EKSU lies within the rainforest zone, while the Ijelu farm is positioned in the savannah forest zone. These locations experience a tropical humid climate characterized by two distinct seasons: a relatively dry period lasting from November to March, and a wet or rainy season prevailing from April to October. The average annual rainfall in the region ranges from 1405 to 2400 mm, with the rainy season accounting for approximately 90% of the total rainfall. The onset of the rainy season typically occurs in April [12]. Regarding temperature, the region experiences a range of 22°C to 33°C. The annual relative humidity stands at 80%, contributing to the overall humid climate. Additionally, the total annual sunshine

hours are approximately 2000 hours, providing ample sunlight for plant growth and development [13].

2.2 Establishment

The field was cleared manually using hoes and cutlasses (minimal tillage). The establishment of the first trial was done in the second week of May, 2019 while that of the second trial was done in the second week of May 2020. Prior to field establishment the garden egg was first raised in the nursery for six weeks. The plot size used for the trial was 3m by 3m. The experiment was laid out in a randomized complete block design (RCBD) and replicated three times. Each replicate has twelve treatments, making a total of thirty-six (36) experimental units. Garden egg seeds at the point of purchase the seeds were already cleaned and ready for use. The seeds were first raised in the nursery for 6 weeks before being transplanted to the field at the rate of 1 (one) seedlings per hole to give a population of thirty-six (36) plants per experimental unit. The first trial was terminated twelve weeks after establishment and the second trial which result is presented was commenced immediately to evaluate the residual effect of the treatments.

2.3 Data Collection

Data collected covers chemical and physical properties of the soils used.

2.4 Soil Sampling

Pre experimental soil samples were collected from the two sites, top soil from each sites was collected and bulked from which a composite sample was collected and analyzed for soil properties. Sampling was also done at the

List 1. Treatment combinations

1.	Control
2.	250 kg NPK
3.	10 tons CD
4.	15 tons CD
5.	10 tons POME
6.	15 tons POME
7.	5 tons POME + 125kg NPK
8.	5 tons POME + 5 tons CD
9.	3.33 tons POME + 3.33 CD + 83.3kg NPK
10.	7.5 tons POME + 125 kg NPK
11.	7.5 tons POME + 5 tons CD
12.	5 tons POME + 3.33 tons CD + 83.3kg NPK

termination of the trial, this time the soils were sampled per experimental units and taken to the laboratory for analysis.

2.5 Soil Analysis

The collected soils were air-dried for about 24 hours, ground and sieved through a 2 mm mesh sieve. Chemical analysis was done to determine the percentage total Nitrogen, available P, exchangeable K, exchangeable cations (Ca, Mg, Na), pH, organic matter content, CEC and base saturation. Soil physical properties such as particle sizes and soil color were also observed.

2.6 Determination of pH

Soil pH was determined using the hydrometer method, 10 grammes of soil was mixed with 20ml of distilled water and stirred at interval for 30 minutes. The pH of the suspension was measured with a pH meter.

2.7 Soil Total Nitrogen (N) and Available Phosphorus

“Percentage total nitrogen was determined by the kjeldahl digestion method. One gramme of the soil sample was digested in conc. H₂SO₄ using selenium tablet as catalyst. The compound formed was then titrated with 0.02 NHCL. Available phosphorus was determined using the Bray and Kurtz (1945) method” [14].

2.8 Exchangeable Cations

Exchangeable cations (K, Ca, Mg and Na) were extracted using 1.0 N ammonium acetate (NH₄OAc) solution at pH 7. About 5 g of soil samples were transferred into a leaching tube and leached with 100 ml of buffered 1.0 N ammonium acetate (NH₄OAc) solution.

2.9 Determination of Soil Potassium and Sodium

Potassium and sodium content in the soil was determined using the flame photometry method. A standard series of potassium and sodium were prepared by diluting both 1000 mg l⁻¹ potassium and sodium solution to 100 mg l⁻¹. This was done by taking a 25 ml portion of each into one 250 ml volumetric flask and made to volume with water. Portions of 0, 5, 10, 15, 20 ml of the 100 mg l⁻¹, standard solution were put into 200 ml volumetric flask respectively. About 100 ml of 1.0 N NH₄OAc solution was added to each flask and made to

volume with distill water. The standard series obtained was 0, 2.5, 5, 7.5, 10 mg l⁻¹ for potassium and sodium. Potassium and sodium were then measured directly by flame photometry at wavelengths of 7665.5 and 589.0 nm respectively.

2.10 Determination of Calcium and Magnesium

“To determine calcium plus magnesium, 25 ml of the extract was transferred into an Erlenmeyer flask. A 1.0 ml portion of hydroxylamine hydrochloride, 1.0 ml of 2.0 percent potassium cyanide buffer (from the burette), 1.0 ml of 2.0 percent potassium ferrocyanide, 10.0 ml ethanolamine buffer and 0.2 ml Eriochrome Black T solution were added. The solution was titrated with 0.01 N EDTA (ethylene diamine tetraacetic acid) to a pure turquoise blue color and the titre values were recorded. The values for calcium were subtracted from this value to get the titre value for magnesium” [14].

Calculation:

$$Ca \text{ (cmol/kg)} = \frac{V1 - V2 \times V4 \times N \times 100 \times mcf}{V3 \times W} \dots \dots \dots \text{equ 2}$$

Where:

- V1 = volume of EDTA required for aliquot sample titration, ml
- V2 = volume of EDTA required for blank titration, ml
- V3 = volume of aliquot taken, ml
- V4 = total volume of original NH₄OAc extract, ml
- N = Normality
- W = Weight of sample taken in g
- Mcf = moisture correlation factor
- 1 mL 0.01N EDTA = 0.2004 mg Ca²⁺ = 0.1216 Mg²⁺

2.11 Determination of Organic Matter

“Soil organic matter content was determined using the walkley-Black oxidation method which measures the active or decomposable organic matter in the soil. The soil sample was ground into fine powder from which 1 g soil sample was taken and placed in a 250 ml conical flask and 10 ml of 0.167 M K₂Cr₂O₇ was added. About 20 ml of conc. H₂SO₄ was rapidly added to the mixture and then swirled gently until the soil and the reagents mixed properly. The mixture is the allowed to cool for about 30 minutes. Three drops

of ferroin indicator was added and titrated against 0.5 M Iron (II) ammonium sulphate. The end product is a brownish red or maroon color solution. Also a blank titration was done without soil" [14].

Calculations:

$$\% \text{ Organic Carbon} = (B-T) \times M \times 0.003 \times 1.33 \times 100/\text{wt}$$

Where:

- B= Blank titre value
- T= Sample titre Value
- M= Molarity of $\text{Fe}(\text{SO}_4)_2$
- Wt= Weight of dried sample
- Percentage Organic Matter is then further calculated as
- %OM= % Organic Carbon x 1.724

2.12 Determination of Textural Class

Particle size analysis was determined using hydrometer method. About 51 grams of air-dried soil was weighed into 250 ml beaker. Then 100 ml of calgon was added and allowed to soak for 30 minutes and transferred to a cup where the suspension is stirred for about 40 seconds. The mixture was subsequently transferred into a 1L sedimentation cylinder and made up to the mark with deionized water. Two readings were taken using the hydrometer, and temperature of the suspension was measured with a thermometer. The initial hydrometer and thermometer readings were taken after agitation of the suspension with a plunger. After the first reading, the suspension was left undisturbed for about 2 hours after which the second reading was taken and recorded. These were then used to compute the percentage sand, silt and clay using the following fomulars.

$$\% \text{ Sand + Clay} = \frac{(R_{40 \text{ sec}} - R_a) + R_c}{\text{Weight of soil}} \times 100 \dots \text{equ 7}$$

$$\% \text{ Clay} = \frac{(R_{2 \text{ hours}} - R_b) + R_d}{\text{Weight of soil}} \times 100 \dots \dots \dots \text{equ 8}$$

Where;

- Ra= 40 seconds blank hydrometer reading
- Rb= 2 hours blank hydrometer reading
- Rc and Rd= 40 Seconds and 2 hours correction factor respectively (Temperature x 0.360).
- Textural class was later determined using the textural triangle.

2.13 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using SPSS version 17 and means were compared with Tukey HSD test to verify significant differences among treatments at 5% probability level. Graphs were generated using Microsoft excel 2010 edition.

3. RESULTS

Table 1 shows the pre experimental soil analysis for the two sites used for the experiment before the first trial. The texture of the two soils is loamy sand, EKSU soil contains 84.40% sand and Ijelu with 74.40% sand. The silt and clay content differ slightly, with EKSU having 8.28% silt and 7.32% clay, while Ijelu contains 12.28% silt and 8.32% clay. The soil pH values also vary, with EKSU having a pH of 5.75 and Ijelu having a pH of 6.80. In terms of organic carbon, EKSU's soil contains 1.06%, while Ijelu's soil has 1.24%. The nitrogen content at both locations is relatively low, with EKSU containing 0.079% nitrogen and Ijelu having 0.13% nitrogen. Phosphorus content in the soil is 12.35 mg/kg for EKSU and 18.56 mg/kg for Ijelu. Regarding exchangeable cations, EKSU's soil has 0.14 cmol/kg, 1.40 cmol/kg, 0.44 cmol/kg, and 0.05 cmol/kg for potassium, calcium, magnesium, and sodium, respectively. On the other hand, Ijelu's soil contains 0.28 cmol/kg, 2.20 cmol/kg, 0.80 cmol/kg, and 0.07 cmol/kg for potassium, calcium, magnesium, and sodium, respectively. The exchangeable acidity in EKSU's soil is 1.20 cmol/kg, while in Ijelu's soil, it is 0.80 cmol/kg.

Table 2 presents the residual effect of the amendment on soil properties, (second trial) The highest PH was 15 tons Cow dung (6.58) while the lowest was 250 Kg NPK (5.72) Organic matter Content was also highest on 15 tons (1.98%) and lowest on Contour (0.68%). Available phosphorus was highest on NPK 250kg treatment (16 mg/kg) while Lowest was Control (8.20 mg/kg). Nitrogen Content was highest on 15 tons cow dung, (0.17%) and lowest on control (0.04%). Highest Calcium Content was recorded on 15 tons Cow dung and 15 tons POME (1.10 cmol/kg), lowest was control (0.90). Highest magnesium content was found on 7.5 tons POME +5 tons cow dung (0.58 cmol/kg) while Lowest value was the Control (0.14 cmol/kg). 10 tons POME had the highest potassium (0.42 cmol/kg) and Sodium Content (0.10 cmol/kg). Lowest potassium and Sodium were recorded on the control (0.06 cmol/kg and 0.04 cmol/kg).

Exchangeable acidity was highest on NPK 250 (1.40 cmol/kg) and lowest on 5 tons POME +3.33 tons Cow dung + 83.8 Kg NPK (0.90 cmol/kg).

Table 3 shows the residual effect of soil amendment on soil properties at Ijelu. pH value was highest in 15 tons Cow dung (6.80) and lowest on NPK 250kg (6.48). Organic matter Content of 1.08% was observed to be highest on treatment. 15 tons cowdung and lowest was found on NPK 250 kg (0.90%). Nitrogen content was highest on 15 tons Cow dung (0.11%) and lowest on Control (0.06%) while available phosphorus was highest on 10 tons POME (16.30 mg/kg) and lowest on Control (8.46 mg/kg) Treatment 10 tons of cow dung gave the highest Calcium content of 1.92 cmol/kg and lowest on Control (1.60 cmol/kg). Magnesium was highest on 10 tons POME, potassium on 15 tons POME as well as Sodium (0.09 cmol/kg). Exchangeable acidity was highest on NPK 250 kg (1.46 cmol/kg). The lowest Value for magnesium is NPK 250kg (0.58 cmol/kg). Lowest value for potassium and sodium is the control with values 0.09 cmol/kg and 0.04 cmol/kg respectively. Exchangeable acidity was lowest on treatment 10 tons POME (0.90 cmol/kg).

4. DISCUSSION

4.1 Residual Effect of Soil Amendments of Soil Properties

The results of this research point our attentions to the fact that soil status over a long period of time is a function of the inputs that goes into it. Palm Oil Mill Effluent (POME) and cow-dung are both organic wastes materials that have potentials to improve soil health and fertility. Soil fertility on the other hand is important in the

performance of crop [15]. This research shows that both POME and cow-dung application to soil either solely or in combination with inorganic fertilizer can influence soil chemical properties positively. Soil acidity was observed to reduce on soils containing POME and cow-dung but found to increase on soil with NPK over time. This could be because of the acid forming nature of fertilizers that contain nitrogen. Nitrogen fertilizers have been known to leave residual hydrogen ions in the soil which results in acidification of the soil. This acid forming property of inorganic fertilizer is absent in fertilizers from organic sources. POME according to the work of Kavitha et al. [16] is alkaline in nature, this can help neutralize the acidity of the soil. Cow-dung on the other hand has been found to have a buffer nature, this attribute helps to maintain soil pH within the range for plant growth [17]. Nutrient elements were also found to increase on soils treated with POME and cow-dung over time. This could be attributed to the fact that these materials are nutrient-rich and contain a high concentration of essential nutrients. Although these elements are important for plant growth and development [18-20] they are often depleted as a result of continuous cultivation of the soil. These materials when added to the soil can help replace the nutrient loss gradually and at the same time ensuring the nutrients are not easily lost unlike the inorganic fertilizer (NPK) that goes into solution once applied to the soil and can easily be lost in moving water. This agrees with the works of Hussain et al. [21] and Ngoma et al. [22] who established that organic materials when applied to soil can help replenish essential nutrients in soil thereby enhancing soil fertility and productivity.

Table 1. Pre-experimental soil analysis

Parameters	EKSU	IJELU
Organic Carbon (%)	1.06	1.24
pH 1:2 (water)	5.75	6.80
Nitrogen (%)	0.09	0.13
Available Phosphorus (mg/kg)	12.35	18.56
Potassium (Cmol/kg)	0.14	0.28
Calcium (Cmol/kg)	1.40	2.20
Magnesium (Cmol/kg)	0.44	0.80
Sodium (Cmol/kg)	0.05	0.07
Exchanged Acidity	1.20	0.80
Particle size analysis		
Sand %	84.40	79.40
Silt %	8.28	12.28
Clay %	7.32	8.32

Table 2. Residual effect of soil amendments on soil properties in EKSU at harvest

S/NO	Treatments	pH (1:2) H ₂ O	OC (%)	AV. P mg/kg	N (%)	Exchangeable bases (Cmol/kg)				E A Cmol/kg
						Ca	Mg	K	Na	
1	Control	5.76e	0.68e	8.20d	0.04d	0.90c	0.14f	0.06e	0.04b	1.20a
2	250 kg N P K	5.72e	0.88d	16.0a	0.09d	1.08a	0.22e	0.10d	0.04b	1.40a
3	10 Tons CD	6.36b	1.02b	12.6b	0.12b	1.08a	0.30d	0.29b	0.09a	1.00b
4	15 Tons CD	6.58a	1.98a	14.8a	0.17a	1.10a	0.42b	0.30b	0.08a	1.00b
5	10 tons POME	6.10c	0.90c	10.20c	0.11b	0.98b	0.38c	0.42a	0.10a	1.20a
6	15 tons POME	6.06d	1.02b	12.80b	0.11b	1.10a	0.46ab	0.30b	0.10a	1.30a
7	5 tons POME + 125 kg NPK	6.12c	0.96c	14.86a	0.08d	0.96b	0.38c	0.24c	0.09a	1.20a
8	5 tons POME + 5 tons CD	6.40a	1.03b	11.40b	0.12b	1.01ab	0.52a	0.30b	0.08a	1.10b
9	3.33 tons POME + 3.33 CD	6.39b	0.96c	9.80c	0.10c	1.04ab	0.32c	0.28bc	0.06b	1.20a
10	7.5 tons POME + 125 kg NPK	6.38b	0.90c	10.24c	0.08d	1.02ab	0.52a	0.22c	0.05b	1.10b
11	7.5 tons POME + 5 tons CD	6.32b	1.08b	11.20b	0.10c	1.00ab	0.58a	0.31b	0.08a	1.20a
12	5 tons POME + 3.33 tons CD + 83.3kg NPK	6.44a	1.02b	9.80c	0.11b	0.96b	0.40b	0.28bc	0.08a	0.90c

Means having the same alphabet are not significantly (<0.05) different from each other

Table 3. Residual effect of soil amendments on soil properties in IJELU at harvest

S/NO	Treatments	pH (1:2) H ₂ O	OC (%)	AV. P mg/kg	N (%)	Exchangeable bases (Cmol/kg)				E A Cmol/kg
						Ca	Mg	K	Na	
1	Control	6.70a	0.92c	8.46e	0.06d	1.60c	0.64d	0.09e	0.04c	1.10c
2	250 kg N P K	6.48d	0.90c	10.20d	0.08c	1.56d	0.58e	0.12d	0.04c	1.40a
3	10 Tons CD	6.76a	1.06a	12.40c	0.10bc	1.92a	0.72c	0.14cd	0.06b	1.00c
4	15 Tons CD	6.80a	1.08a	14.20b	0.11a	1.90a	0.90a	0.20a	0.06b	1.10c
5	10 tons POME	6.70a	1.02b	16.30a	0.10bc	1.83a	0.98a	0.18b	0.07b	0.90d
6	15 tons POME	6.64b	1.05a	12.40c	0.10bc	1.40e	0.90a	0.26a	0.09a	1.20b
7	5 tons POME + 125 kg NPK	6.58c	0.98bc	10.90d	0.09c	1.60c	0.82b	0.18b	0.07b	1.30a
8	5 tons POME + 5 tons CD	6.70a	1.04a	12.30c	0.10bc	1.70b	0.78bc	0.17b	0.05c	1.10c
9	3.33 tons POME + 3.33 CD	6.62b	1.02b	13.48c	0.10bc	1.68bc	0.69cd	0.16bc	0.07b	1.20b
10	7.5 tons POME + 125 kg NPK	6.69b	1.00bc	14.50b	0.10bc	1.37f	0.66d	0.14c	0.05c	1.40a
11	7.5 tons POME + 5 tons CD	6.70a	1.02b	16.20a	0.08c	1.40e	0.70c	0.15c	0.04c	1.10c
12	5 tons POME + 3.33 tons CD + 83.3 kg NPK	6.60b	0.99bc	12.40c	0.07d	1.29g	0.62d	0.12d	0.03d	1.10c

Means having the same alphabet are not significantly (<0.05) different from each other

The high organic matter content contained the soils treated with POME and cow-dung could also be attributed to the fact that these material are carbon rich materials and are high in organic matter content. Organic matter serves also as a vital component in soil structure formation, moisture retention, and nutrient holding capacity [23], (Singh et al., 2021). The latter is a major feature that helps to reduce nutrient losses from the soil. Furthermore high organic matter helps to improve soil aggregation, thereby enhancing soil porosity, infiltration and aeration all of which are vital for proper root growth and overall health of soil ecosystem [24,25,26].

5. CONCLUSION

In conclusion, soil sustainability and productivity is more attainable when amendments have a long term positive effect on soil. POME and cow-dung can offer a long-term benefit to soil, including nutrient enrichment, organic matter addition to the soil, promote microbial activities, regulate soil pH and also ensure environmental stability. Inorganic NPK fertilizer on the other hand might have a short term benefit but could leave a residual negative effect on the soil particularly when used continuously. By effectively utilizing these organic waste materials, farmers and land managers can improve soil fertility, productivity and overall ecosystem health in a sustainable manner.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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