



The impact of Palm Oil Mill Effluent (POME) application on the rhizosphere heterotrophic population, nitrogen fixing bacteria and enzyme activity of an agricultural soil

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Abstract

Palm Oil Mill Effluent (POME) is considered an efficient soil conditioner due to its high organic contents but the presence of phenol compounds limits their widespread use in agriculture. In the present study, POME was subjected to both aerobic and anaerobic decomposition to reduce its organic strength and degrade its toxic fractions. It was then applied to support *Arachis hypogea* grown in a green house. The rhizosphere environment was monitored for changes in total bacterial counts, nitrogen fixing bacteria and enzyme activities. Results show that the bacteria counts were higher ($302.0 \pm 1.41 \times 10^5$ cfu/g) in mesocosms treated with 100% aerobically digested POME but lower ($280.0 \pm 1.41 \times 10^5$ cfu/g) in the batch treated with anaerobically digested POME. Nitrogen fixing bacteria was $106.0 \pm 2.83 \times 10^2$ cfu/g in mesocosms treated with aerobically fermented POME compared to $64.0 \pm 2.83 \times 10^2$ cfu/g from the counterpart treated with anaerobic digests. Dehydrogenase activity was highest (52.83 ± 0.01 mg TPFg⁻¹24h⁻¹) in soils treated with aerobically digested POME but not higher (48.65 ± 0.03 mg TPFg⁻¹24h⁻¹) than the treatments which received the anaerobically modified effluent. Acid phosphatase activity was lower (201.0 ± 0.28 IU PNP/g/ 24h) in the soils treated with aerobically modified POME but higher (213.5 ± 0.42 IU PNP/g/24h) in batches dosed with anaerobically treated effluent. Urease activity increased to peak level (2.437 ± 0.004 Ppm NH₄⁺-N /g/24h) in the 4th week but dropped to its final concentration of 1.615 ± 0.001 Ppm NH₄⁺-N /g/24h) in soils treated with anaerobically digested POME. Data from mesocosms treated with the anaerobic digests were lower (2.149 ± 0.004 and 1.500 ± 0.014 ppm NH₄⁺-N /g/ 24h respectively).

KeyWords: Palm oil mill effluent, phenol, dehydrogenase, phosphatase, urease.

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Introduction

Today, many industrial activities which brought improved living conditions to man have left behind enormous wastes which pose serious dangers to human health and life as well as to the overall well-being of the ecosystem (Olorunfemi et. al., 2008). The global production of palm oil for instance is growing at a very high rate and the pollution caused by the reckless discharge of the untreated effluent has raised serious environmental concerns. Palm oil processing is

carried out using large quantities of water in mills where oil is extracted from the palm fruits. During the extraction process, 50% of the water results in wastes called palm oil mill effluent (POME). It is estimated that for every 1 tonne of crude palm oil produced, 5.0 - 7.5 tonnes of water end up as POME (Wu et. al., 2009). POME contains high organic content, substantial amounts of plant materials and could be a low cost source of plant nutrients (Okwute and Isu, 2007). It is generally believed that the

presence of phenols and other organic acids in POME are responsible for their phytotoxic effect and antibacterial activity (Capasso et. al., 1992; Pascual et. al., 2007). However, according to Piotrowska et. al. (2006), the polyphenolic fraction degrades after sometime and transforms into humic substances which could be applied in soil conditioning. Treated POME has an extremely high content of degradable organic matter, which is due in part to the presence of unrecovered palm oil and the activity of microorganisms degrading the toxic components (Ahmad et. al., 2003).

Microorganisms in the soil are responsible for nitrogen fixation, assimilation, and degradation of organic residues to release nutrients (Nwuche and Ugoji, 2010). The soil microbial community therefore is the primary mediators of key biological processes in soil (Li et. al., 2009). These processes control ecosystem carbon and nitrogen cycling and play important roles in maintaining soil ecosystem quality and functional diversity (Schloter et. al., 2003). Nitrogen is a common soil nutrient element required in large quantity by plants. The growth of higher plants in many environments is limited by nitrogen supply. Nitrogen is largely made available to plant in form of ammonium or nitrate ion by the activities of soil microorganisms through the process of nitrogen fixation. These microorganisms are the nitrifying bacteria (John et. al., 2011). The root system of higher plants especially legumes is associated not only with inanimate environment comprising organic and inorganic substances but also a vast community of metabolically active microorganisms referred to as rhizosphere organisms. Interactions between the macro and micro-organisms in the rhizosphere can be of considerable significance for crop production and soil fertility. The microbes depend on the legumes for the basic nutrients needed to sustain their life functions. The nitrogen-fixing capacity of legumes stabilizes the soil nitrogen and organic carbon content in the root zone to a greater extent than crops grown alone (John et.

al., 2011).

Orji et. al. (2006) observed that soils receiving fresh POME discharges had very scanty microbial population and diversity. Thus the ecosystem changes in response to the presence of contaminants or pollutants like POME. These soil microorganisms also harbor enzymes whose activities ensure the continuity of elemental cycles in nature. The enumeration of microorganisms and assessment of the activity of the soil enzymes provide an integrative measure of the state of health of any soil (Li et. al., 2005). Presently, little information is available on the impact of POME on the rhizosphere heterotrophic and nitrogen-fixing populations of an agricultural soil. This is because, POME produced by the numerous small-scale traditional operators undergo little or no treatment and is usually discharged into the surrounding water or agricultural fields where they constitute potent dangers to resident flora and fauna. In this report, the impact of the effluent on soil rhizosphere micro flora and activities of key enzymes are evaluated.

Materials and methods

Sample collection and preparation

Soil samples were collected at 0-15 cm depth from a field plot within the premises of the University of Nigeria, Nsukka using a soil auger. The soils were put into sterile containers and taken away for processing. In the laboratory, the soil was sieved using a 2 mm sieve, sorted to remove stones and plant debris and then thoroughly mixed to ensure uniformity.

Precisely 2 kg quantity of soil was transferred into four separate replicate plastic pots or mesocosms (per treatment) for plant cultivation. The dimensions of each pot were 15 cm (height) by 25 cm (diameter). The containers were perforated at the bottom to facilitate drainage. Each mesocosm contained a total plant density of five and were maintained in a green house. The groundnut seeds (*Arachis hypogea*) used in the planting experiment were purchased from Ogige market Nsukka and

taxonomically identified at the Crop Science Department, University of Nigeria Nsukka. Fresh POME samples were collected in clean plastic containers from local palm oil producers within Nsukka metropolis.

The POME was homogenized by passing through a 2 mm sieve to remove the different plant fractions before subjecting them to aerobic and anaerobic digestion.

For the aerobic treatment, the effluent was left to ferment at $28 \pm 2^\circ\text{C}$ in sterile 20 L open container and periodically turned to promote mixing. Anaerobic digestion was carried out in a single stage laboratory scale digester having hydraulic retention time of 20 days. The system involves re-circulating the leachate at the top of the reactor for mixing to occur. The digester was hermetically sealed to maintain anaerobic condition and an outlet was provided for the release of waste gases. The end of the outlet was dipped into water through a network of interconnected tubes to prevent the entry of air by reverse movement. Both the aerobic and anaerobically digested POME were maintained at 4°C to forestall further microbial decomposition.

Following germination, the moisture content of the mesocosms were maintained to 50% water holding capacity (WHC) by the application of different concentrations (25, 50, 75 and 100%) of the digested POME. Distilled water (50 ml/200g) was used for the control experiment. When the plants has developed 2-3 leaves and attained a height of 4-5 cm, the rhizosphere of two plants from each treatment were examined each week for changes in the total bacteria density, nitrogen-fixing bacteria and enzyme activity. Average of the recorded results \pm standard error of means was reported.

Enumeration of the total heterotrophic nitrogen-fixing bacteria

The population of bacteria around the rhizosphere of each plant was enumerated by the viable plate count method (Zuberer, 1994) while that of the nitrogen fixing bacteria was carried out on Ashby Mannitol Agar according to the descriptions of John et. al. (2011).

Analysis of soil enzyme activities

The dehydrogenase assay method as described by Tabatabai (1997) was used. The assay

involved colorimetric estimation of 2, 3, 5-triphenyl formazan (TPF) produced by the reduction of 2, 3, 5-triphenyltetrazolium chloride/tetrazolumtrichloride (TTC) by soil microorganisms. The acid and alkaline phosphatases were determined according to the method of Tabatabai (1982) and depended on the colorimetric estimation of the p-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 6.5 for acid phosphatase activity and pH 11.5 for alkaline phosphatase activity) sodium p - nitrophenyl phosphate solution and toluene. Urease was measured by the method of Nannipieri et. al. (1980). The method is based on the determination of ammonia released after incubation of soil samples with urea solution for 2 h at 37°C .

Analytical methods

The soil physico-chemical properties were determined by AOAC (2005). The pH, oil and grease (O and G), and total suspended solids (TSS) were determined by Standard Methods (APHA, 2005). Chemical oxygen demand (COD) was measured by the Hach's Spectrophotometric method (DR/4000, Hach Co., Ltd., Tokyo). Phenol was determined by the phenol test kit (Wako Pure Chemical Industries, Osaka) and the emerging colour from the test reaction matched against a standard to determine the concentration of phenol in the sample

Statistical analysis

Statistical analysis of the results was carried out using Post Hoc (Turkey) test analysis of variance (ANOVA). SPSS statistical software version 23 was used to assess the level of significance at 95% and reported as mean \pm standard deviation (SD) of triplicate experiments.

Results and Discussion

Characteristics of soil and POME used in the study

The physico-chemical properties of the soil used in the study are presented in Table 1. The soil was sandy-loam, of varied particle sizes and pH of 5.4. Many soils are affected by acidic conditions due to heavy fertilization and acid rain (Onyia et. al., 2001 and Nwoko et. al., 2010).

The organic carbon and organic matter content were 1.64 and 2.82% respectively while the moisture content was 6.6%. The presence of nitrogen (0.126%), ammonia (0.136%), nitrate (0.074%) and nitrite (0.042%) were confirmed while electrical conductivity was 3.0 mS/cm. In Table 2, the characteristics of POME used in the study were highlighted. The raw POME had a pH of 4.4.

Table 1: Physicochemical properties of soil used in the study

Parameters (%)	Soil
Texture class	Sandy- loam
Particle Size:	
Clay	14
Silt	3.0
Sand (Fine)	38
Sand (Coarse)	45
pH	5.4
Organic Carbon	1.64
Organic matter	2.82
Nitrogen	0.126
Ammonia	0.136
Nitrate	0.074
Nitrite	0.042
Available Phosphorus*	25.18
Magnesium	3.010
Available Potassium*	3.5
Electrical Conductivity**	3.0
Moisture content	6.6

*ppm **mS/cm

Table 2 : Characteristics of POME used in the study

Parameters (mg/l)	Raw effluent	Aerobically Digested	Anaerobically digested
pH	4.3±0.2	4.8 ±0.4	5.2 ±0.4
Oil and Grease	3200±200	1600±100	2800±150
COD	68,500±300	30,825±250	4,150±300
Total suspended solids (TSS)	25,000±400	9,500±150	1,270±400
Phenol	100±10	ND	ND

*Not measured in mg/L

The oil and grease (3200±200 mg/L) and chemical oxygen demand (68,500±300 mg/L) were found to agree with earlier reports (Nwuche et. al., 2013; Nwuche et. al., 2014). The total suspended solid was 25,000±400 mg/L. Of particular note was the presence of phenol (100±10 mg/L). The phytotoxic and antibiotic property associated with POME is often due to

the phenol components (Wattanapenpaiboon and Wahlqvist, 2003). Some parameters changed in response to aerobic and anaerobic degradation. For instance, pH increased to 4.8 and 5.2 respectively. In the aerobically digested POME, oil and grease decreased by 48% (3200 to 1,650 mg/L) while COD dropped by 55% (68,500 to 30,825 mg/L). The TSS diminished by 62% (25,000 to 9,550 mg/L) while phenol disappeared. The O and G in the anaerobically treated POME declined by only 13% (3,200 to 2,800 mg/L) while the COD and TSS lost 94% (68,500 to 4,150 mg/L) and 95% (25,000 and 1,270 mg/L) respectively. Phenol was eliminated by the treatment.

Effect of Treated POME on soil pH

The pH changes of the rhizosphere soil after treatment with different POME concentrations are shown in Table 3.

The pH of the control treatment remained stable but in the mesocosms treated with the digested effluents, significant (p < 0.05) increase in pH occurred after the 4th week in the pots treated with lower POME (25 and 50%) concentrations. The pH of the soil samples treated with aerobically digested POME increased to 6.1±0.07 and 5.7±0.07 respectively. By the 5th week, it further rose to 6.2±0.07 and 5.9±0.07. At higher POME concentrations (75 and 100%) pH increase in soil was lower. Initially (week 1), pH of soil samples receiving 75% and 100% POME was 5.0±0.07 and 4.8±0.07 respectively perhaps due to the acidity remaining in the digested effluents. However, by the 4th week of treatment, pH has risen to 5.5±0.07 and 5.2±0.00 respectively and 5.7±0.07 and 5.5±0.07 after the 5th week. The same trend was observed in the mesocosms treated with the anaerobically digested POME except that the increment in pH of soil treated with the more diluted POME (25 and 50%) was lower compared with the aerobically digested applications. The increase in the pH of soil treated with the digested POME could be as a result of nitrification activities of the NH₄-N compounds (Pascual et.

al., 2007) which yielded ammonia causing the increase in the pH.

In the present study, this process proceeded at faster rate at low POME concentration (25 and 50%) resulting in higher pH increase than at higher POME concentrations (75 and 100%). Perhaps the high organics present in the effluent required extended period for complete degradation. The application of organic materials has been reported to promote soil fertility by improving soil pH more than soils treated with inorganic fertilizers (Adeniyani et. al., 2011). Soil pH therefore, is one of the principal factors affecting nutrient availability to plants (Buri et. al., 2005). At pH less than 5.5, low levels of

Table 3: Effect of different POME concentrations on pH changes in the rhizosphere of an agricultural soil after weeks of treatment

Week	POME Concentration (%)								
	Aerobically Digested POME				Anaerobically Digested POME				
	Control	25	50	75	100	25	50	75	100
1	5.4±0.14 ^{ab}	5.3±0.14 ^{ab}	5.2±0.07 ^{ab}	5.0±0.07 ^{ab}	4.8±0.07 ^a	5.2±0.07 ^{ab}	5.0±0.07 ^{ab}	4.9±0.07 ^{ab}	4.7±0.07 ^a
2	5.5±0.14 ^{ab}	5.4±0.14 ^{ab}	5.3±0.07 ^{ab}	5.1±0.07 ^{ab}	4.9±0.07 ^{ab}	5.3±0.07 ^{ab}	5.2±0.07 ^{ab}	5.0±0.07 ^{ab}	4.8±0.07 ^a
3	5.5±0.14 ^{ab}	5.7±0.14 ^{ab}	5.5±0.07 ^{ab}	5.3±0.07 ^{ab}	5.1±0.07 ^{ab}	5.5±0.07 ^{ab}	5.3±0.07 ^{ab}	5.1±0.07 ^{ab}	4.9±0.07 ^a
4	5.5±0.14 ^{ab}	6.1±0.07 ^{ab}	5.7±0.07 ^{ab}	5.5±0.07 ^{ab}	5.2±0.07 ^{ab}	5.8±0.14 ^{ab}	5.6±0.07 ^{ab}	5.3±0.07 ^{ab}	5.1±0.07 ^a
5	5.5±0.14 ^{ab}	6.2±0.07 ^{ab}	5.9±0.07 ^{ab}	5.7±0.07 ^{ab}	5.5±0.07 ^{ab}	5.9±0.07 ^{ab}	5.7±0.07 ^{ab}	5.4±0.07 ^{ab}	5.2±0.07 ^a

KEY: Alphabets in capital letters = Mean difference significant between various %concentration of POME treatment

Alphabets in small letters = Mean difference significant within a particular %concentration of POME treatment

NOTE: Same capital letters are not statistically different among treatments by the Turkey test (p<0.05) and small lower cap letters are not statistically different among weeks by the Turkey test (p<0.05) ± standard deviation.

mineral elements are found in the soil. In their study, Nwoko et. al. (2010) observed increase in soil available N, P and K and decrease in acidity of POME due to biodegradation of the organic materials.

Effect of POME Treatment on the total rhizosphere heterotrophic counts

The changes in the total heterotrophic counts due to the effect from POME application are presented in Table 4.

Table 4: Changes in the total heterotrophic bacteria count ($\times 10^5$ cfu/g) in the rhizosphere of *Arachis hypogaea* treated with aerobically and anaerobically fermented POME

Week	Control	POME Concentration (%)							
		Aerobically Digested POME				Anaerobically Digested POME			
		25	50	75	100	25	50	75	100
1	25.00±7.07 ^{ab}	11.50±0.71 ^{ab}	37.50±0.71 ^{ab}	46.00±5.66 ^{ab}	56.00±5.66 ^{ab}	7.00±1.41 ^{ab}	21.00±1.41 ^{ab}	24.50±0.71 ^{ab}	28.00±1.41 ^{ab}
2	32.00±2.82 ^{ab}	100.50±0.71 ^{ab}	113.00±4.24 ^{ab}	137.50±4.92 ^{ab}	186.50±3.54 ^{ab}	70.50±0.71 ^{ab}	75.50±7.07 ^{ab}	99.00±1.41 ^{ab}	112.00±2.82 ^{ab}
3	36.00±0 ^{ab}	114.00±5.66 ^{ab}	127.00±4.24 ^{ab}	136.00±5.66 ^{ab}	220.00±5.66 ^{ab}	100.00±0 ^{ab}	111.50±9.19 ^{ab}	123.00±1.41 ^{ab}	159.00±1.41 ^{ab}
4	33.50±2.12 ^{ab}	188.00±5.66 ^{ab}	217.00±1.41 ^{ab}	262.00±5.66 ^{ab}	288.00±11.31 ^{ab}	182.00±2.82 ^{ab}	192.00±5.66 ^{ab}	220.00±2.82 ^{ab}	264.00±2.82 ^{ab}
5	34.00±4.24 ^{ab}	196.50±2.12 ^{ab}	215.50±7.78 ^{ab}	269.50±10.61 ^{ab}	302.00±1.41 ^{ab}	188.00±2.82 ^{ab}	199.50±0.71 ^{ab}	222.50±2.12 ^{ab}	280.00±1.41 ^{ab}

KEY:

Alphabets in capital letters = Mean difference significant between various %concentration of POME treatment

Alphabets in small letters = Mean difference significant within a particular %concentration of POME treatment

NOTE: Same capital letters are not statistically different among treatments by the Turkey test (p<0.05) and small lower cap letters are not statistically different among weeks by the Turkey test (p<0.05) ± standard deviation.

Data showed there were increase in bacterial counts with increase in POME concentrations but the total microbial counts obtained from the rhizosphere samples polluted with the aerobically digested POME was significantly higher (p < 0.05) than the counts obtained in the samples treated with anaerobically digested POME. In week 1, the total bacterial counts in the low POME concentrations (25 and 50%) were 11.50±0.71 and 37.50±0.71 $\times 10^5$ cfu/g compared to 46.00±5.66 and 56.00±5.60 $\times 10^5$ cfu/g obtained at higher POME (75 and 100%) concentrations. In the anaerobically digested treatments, the total bacterial counts in the mesocosms treated with low POME concentrations were 7.00±1.41 and 21.00±1.41 $\times 10^5$ cfu/g respectively while at higher concentrations, the counts increased to 24.50±0.71 and 28.00±1.41 $\times 10^5$ cfu/g respectively. The same pattern of increment in bacterial counts was observed up till the end of experiment. In fact by the 5th week, population counts in the low POME (25 and 50%) treated mesocosms had increased to 196.50±2.12 and 215.50±7.78 $\times 10^5$ cfu/g compared to 188±2.83 and 199.50±0.71 $\times 10^5$ cfu/g in the anaerobically digested POME rose to 269.50±10.61 and 302.00±1.41 $\times 10^5$ cfu/g while those from the anaerobically digested treatments were 222.50±2.12 and 280.00±1.41 $\times 10^5$ cfu/g respectively.

The increase in microbial population observed in rhizosphere samples treated with POME might result from the rich organic content and the presence of easily degradable materials, which promoted rapid growth of both the soil native and exogenous microorganisms in the POME (Clemente et. al., 2006). Fermented POME contains free fatty acids, starch, proteins and their degradation products (Bek-Nielsen et. al., 1999). However, the heterotrophic counts in soils taken from mesocosms treated with aerobically fermented POME were higher ($p < 0.05$) compared to those treated with anaerobically fermented POME perhaps due to cross contamination and the invasion and proliferation of many different organisms during the 'open' aerobic digestion treatment. During this process, diverse community of microorganisms thrives in succession to participate in the breakdown of complex organic materials in the POME into simple and easily utilizable forms. This opinion is supported by Okwute and Isu (2007). In the anaerobically digested POME, the treatment conditions are different and selective resulting in decreased populations and reduced microbial diversity. When biological wastes are subjected to anaerobic decomposition, they naturally yield a variety of organic acids (Lam and Lee, 2011). The process of anaerobic digestion of POME involves a sequence of metabolic reactions which include Hydrolysis, acidogenesis, acetogenesis and methanogenesis (Demirel and Scherer, 2008).

During hydrolysis, organic polymers (i.e. carbohydrates, proteins and lipids) are converted to their respective monomers. This conversion is usually mediated by several hydrolytic enzymes such as cellulases, xylanases, amylases, lipases and proteases produced by the microbes (Weiland, 2010). In acidogenesis, the organic monomers produced in the earlier hydrolytic phase are utilized as substrates by anaerobic acidogenic bacteria to produce organic acids, hydrogen and carbon dioxide. The acetogenetic phase is characterized by the formation of several organic acids such as

acetic, propionic, butyric and lactic acids as well as carbon dioxide and hydrogen. Hydrogen and carbon dioxide are utilized by hydrogenotrophic methanogens while the complex intermediary compounds are even further converted to simpler organic acids before being used by acetotrophic or acetoclastic methanogens in the last stage of the overall process to produce methane (CH_4) and carbon dioxide (Poh and Chong, 2009).

Effect of POME treatment on the rhizosphere nitrogen fixing bacteria

In Table 5, the effect of the fermented POME on the populations of rhizosphere nitrogen fixing bacteria is presented.

Table 5: Changes in the total nitrogen fixing bacteria count ($\times 10^2$ /g) in the rhizosphere of *Arachis hypogea* treated with aerobically and anaerobically fermented POME

Week	Control	POME Concentration (%)							
		Aerobically Digested POME				Anaerobically Digested POME			
		25	50	75	100	25	50	75	100
1	30.0±2.83 ^{Ab}	68.0±2.83 ^{Bb}	36.0±2.83 ^{Ab}	31.0±2.83 ^{Ab}	28.0±1.41 ^{Ab}	44.0±1.41 ^{Bb}	35.0±1.41 ^{Bb}	28.5±0.71 ^{Ab}	23.0±1.41 ^{Ab}
2	30.5±3.54 ^{Ab}	45.0±5.66 ^{Bb}	33.0±4.24 ^{Ab}	27.0±2.83 ^{Ab}	14.0±5.66 ^{Ab}	31.0±4.24 ^{Bb}	25.5±0.71 ^{Ab}	21.5±2.12 ^{Ab}	9.0±1.41 ^{Ab}
3	34.0±5.66 ^{Ab}	107.5±2.12 ^{Bb}	79.0±2.83 ^{Bb}	63.0±5.66 ^{Bb}	29.5±4.95 ^{Ab}	100.5±2.12 ^{Bb}	61.5±2.12 ^{Bb}	31.0±4.24 ^{Ab}	19.0±8.49 ^{Ab}
4	34.0±2.83 ^{Ab}	142.0±5.66 ^{Bb}	120.0±8.49 ^{Bb}	101.0±9.90 ^{Bb}	84.0±2.83 ^{Bb}	118.0±2.83 ^{Bb}	86.0±5.66 ^{Bb}	59.5±0.71 ^{Ab}	27.0±4.24 ^{Ab}
5	36.0±1.41 ^{Ab}	141.5±3.54 ^{Bb}	128.5±0.71 ^{Bb}	115.0±1.41 ^{Bb}	106.0±2.83 ^{Bb}	131.0±5.66 ^{Bb}	118.5±4.95 ^{Bb}	85.0±1.41 ^{Ab}	64.0±2.83 ^{Bb}

KEY:

Alphabets in capital letters shows significant difference between various %concentration of POME treatment

Alphabets in small letters shows significant difference within a particular %concentration of POME treatment

NOTE: Same capital letters are not statistically different among treatments by the Turkey test ($p < 0.05$) and small lower cap letters are not statistically different among weeks by the Turkey test ($p < 0.05$) ± standard deviation.

Results indicate that increase in the concentration of POME applied to the respective mesocosms resulted in decrease in the number of nitrogen fixing bacteria in the rhizosphere. In week 1 for instance, the total populations of nitrogen fixing bacteria in the mesocosms treated with low concentrations (25 and 50%) of aerobically digested POME were 68 ± 2.83 and $36.0 \pm 2.83 \times 10^2$ cfu/g respectively but in the high concentration (75 and 100%) treatment, the counts decreased to 31.0 ± 2.83 and $28.0 \pm 1.41 \times 10^2$ cfu/g respectively. However, within each treatment, there was significant

increase in the populations of the diazotrophs as time progressed. By the 5th week of treatment, the populations of nitrogen fixing bacteria in the mesocosms receiving low concentrated treatments (25 and 50%) advanced to 141.5±3.54 and 128.5±0.71 × 10² cfu/g respectively while in the more concentrated (75 and 100%) treatments, increase in the counts to 115.0±1.41 and 106.0±2.83 ×10² cfu/g were observed respectively. The anaerobically digested treatments did not differ from the pattern already described except that the populations of diazotrophs were significantly lower (p < 0.05) than data obtained from the mesocosms treated with aerobically digested effluent. In week 1, it can be seen that the populations of nitrogen fixers in the rhizosphere were 44.0±1.41 and 35.0±1.41 × 10² cfu/g in the mesocosms treated with 25 and 50% POME respectively. With increase in the concentration of the effluent (75 and 100%), the bacterial counts declined to 28.5±0.71 and 23.0±1.41 × 10² cfu/g respectively. At the end of the experiment (5th week), the diazotrophs in the former had increased to 131.0±5.66 and 118.5±4.95 × 10² cfu/g respectively while in the latter (75 and 100%), significant increment in counts up to 85.0±1.41 and 64.0±2.83 × 10² cfu/g was achieved. The factors responsible for the decrease in number of nitrogen fixing bacteria at Higher (75% and 100%) POME concentration may include decreased aeration and moisture due to the higher oil content of the effluent. The decomposition of the organic materials in POME by soil microbes often lead to oxygen depletion which result in diminished aerobic activity (Nwoko et. al., 2010). The rate of nitrogen fixation is also affected by soil moisture. There is usually a decrease in the moisture content of POME impacted soil. This is because the water fraction tends to get quickly absorbed into the soil before the heavier oil fractions which migrate slowly afterwards. During this movement, oil displaces the water and then occupies the soil interstices resulting in reduced moisture content. Therefore, contaminated soils do not only have excessive bio-load but may be

physiologically "dry" even when the soil is wet (John et. al., 2011).

Effect of POME treatment on the soil enzyme activity

Soil dehydrogenase increased with increase in POME concentrations (Table 6)

Table 6: Effect of different POME concentrations on soil Dehydrogenase activity (mg TPFg⁻¹24h⁻¹) after several weeks

Week	POME Concentration (%)								
	Control	Aerobically Digested POME				Anaerobically digested POME			
		25	50	75	100	25	50	75	100
1	12.99±0.04 ^{Ab}	14.04±0.01 ^{2a}	15.30±0.14 ^{2a}	18.91±0.00 ^{2a}	24.48±0.03 ^{2a}	13.03±0.01 ^{Ab}	14.91±0.03 ^{2a}	16.82±0.03 ^{2a}	28.87±0.01 ^{2a}
2	13.03±0.14 ^{Ab}	16.60±0.14 ^{2a}	24.91±0.01 ^{2a}	30.83±0.13 ^{2a}	37.34±0.03 ^{2a}	14.94±0.09 ^{2a}	24.58±0.21 ^{2a}	29.10±0.14 ^{2a}	32.82±0.11 ^{2a}
3	13.97±0.03 ^{Ab}	21.96±0.06 ^{2a}	34.15±0.07 ^{2a}	38.02±0.01 ^{2a}	43.15±0.21 ^{2a}	21.27±0.01 ^{2a}	31.97±0.06 ^{2a}	34.54±0.08 ^{2a}	39.91±0.03 ^{2a}
4	13.85±0.01 ^{Ab}	33.01±0.01 ^{2a}	39.35±0.01 ^{2a}	43.56±0.04 ^{2a}	48.12±0.01 ^{2a}	22.00±0.14 ^{2a}	33.05±0.02 ^{2a}	38.83±0.03 ^{2a}	43.48±0.01 ^{2a}
5	13.97±0.01 ^{Ab}	36.17±0.01 ^{2a}	43.32±0.01 ^{2a}	44.89±0.01 ^{2a}	52.83±0.01 ^{2a}	27.52±0.03 ^{2a}	36.98±0.01 ^{2a}	42.96±0.01 ^{2a}	48.65±0.03 ^{2a}

KEY:

Alphabets in capital letters = Mean difference significant between various %concentration of POME treatment

Alphabets in small letters = Mean difference significant within a particular %concentration of POME treatment

NOTE: Same capital letters are not statistically different among treatments by the Turkey test (p<0.05) and small lower cap letters are not statistically different among weeks by the Turkey test (p<0.05) ± standard deviation.

Significant increase (p < 0.05) was equally observed within each treatment as the weeks progressed but there were no difference in activity in soils treated with either the aerobically or anaerobically digested effluents. Dehydrogenase activity increased from 14.04±0.01 mg TPFg⁻¹24h⁻¹(25% POME) in soils treated with aerobically digested POME to a final concentration of 24.48±0.03 mg TPFg⁻¹24h⁻¹in soils receiving the most concentrated (100%) effluent. In the mesocosms treated with anaerobically digested POME, identical data was obtained. The activity in the soils treated with 25% POME was 13.03±0.01 mg TPFg⁻¹24h⁻¹ but increased to 28.87±0.01 mg TPFg⁻¹24h⁻¹ at the mesocosms treated with 100% POME. The highest activity however occurred in the respective treatments after 5 weeks of experiment. The dehydrogenase in the mesocosms treated with 25% POME increased to 36.17±0.01 mg TPFg⁻¹24h⁻¹; on the other hand, activity in the soils treated with 100% POME rose to 52.83±0.01 mg TPFg⁻¹24h⁻¹while in the mesocosms treated with anaerobically digested POME; the activity was 48.65±0.03 mg TPFg⁻¹24h⁻¹. The control treatment was unaffected.

Soil microbiological and biochemical properties are known to respond rapidly to changes that occur in soil, thereby providing information on the state of soil health (Smith et. al., 1993). The addition of fermented POME to the soil increased the organic matter content which promoted biodegradation by microorganisms. During this process, there was rapid multiplication of the microbes which positively affected the activity of the enzyme. Ranamukharachchi and Doi (2009) had correlated soil dehydrogenases with increased bacterial populations in soils. Thus the soil total dehydrogenase reflects the workings of a group of enzymes that are present in soil (Dick, 1996) and their assay offers a continuous measure of soil microbial activity. The study of the activity of soil enzymes is important because they indicate the potential of a soil to carry out specific biochemical roles, particularly the maintenance of soil fertility (Nwoko et. al., 2010). Soil enzymes catalyze the release of nutrients by means of organic matter degradation; however soil dehydrogenases are one of the main components of the soil enzyme system participating in and assuring the correct sequence of all biochemical roles in the biogeochemical cycles (Kumar et. al., 2013). Therefore, its measure serves as an integrative assessment of soil quality.

Phosphatase catalyses the hydrolysis of organic phosphorus compounds to phosphates (Mudge et. al., 2002). From the results shown in Table 7, there was significant ($p < 0.05$) increase in acid phosphatase in the soils treated with either the aerobically or the anaerobically digested POME at high concentrations.

Table 7: Effect of different POME concentrations on the soil acidic phosphatase (IU PNP/g/ 24h) activity after several weeks

Week	POME Concentration (%)								
	Control	Aerobically Digested POME				Anaerobically Digested POME			
		25	50	75	100	25	50	75	100
1	63.5±0.28 ^{2a}	48.5±0.03 ^{4a}	56.0±0.01 ^{3a}	86.5±0.28 ^{2a}	164.5±0.14 ^{2a}	77.5±0.21 ^{2a}	89.0±0.03 ^{2a}	110±0.00 ^{2a}	190.0±0.28 ^a
2	64.0±0.06 ^{2a}	53.6±0.00 ^{4a}	59.0±0.03 ^{3a}	90.0±0.14 ^{2a}	168.05±0.08 ^{2a}	81.5±0.28 ^{2a}	94.0±0.04 ^{2a}	117.5±0.14 ^{2a}	194.0±0.14 ^a
3	64.5±0.28 ^{2a}	62.05±0.08 ^{4a}	68.0±0.03 ^{3a}	94.5±0.28 ^{2a}	172.5±0.14 ^{2a}	83.3±0.18 ^{2a}	113.0±0.14 ^{2a}	122.0±0.10 ^{2a}	199.0±0.28 ^a
4	63.0±0.14 ^{2a}	60.5±0.28 ^{4a}	66.5±0.14 ^{3a}	93.5±0.42 ^{2a}	173.0±0.14 ^{2a}	83.5±0.28 ^{2a}	110.5±0.14 ^{2a}	125.5±0.14 ^{2a}	198.5±0.28 ^a
5	64.5±0.42 ^{2a}	61.5±0.14 ^{4a}	69.5±0.42 ^{3a}	101.5±0.28 ^{2a}	201.0±0.28 ^{2a}	83.5±0.14 ^{2a}	118.5±0.28 ^{2a}	138.5±0.14 ^{2a}	213.5±0.42 ^a

KEY:

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Alphabets in small letters = Mean difference significant within a particular %concentration of POME treatment

NOTE: Same capital letters are not statistically different among treatments by the Turkey test ($p < 0.05$) and small lower cap letters are not statistically different among weeks by the Turkey test ($p < 0.05$) ± standard deviation.

By the 5th week, the lowest phosphatase 61.5±0.14 IU PNP /g/ 24h activity was observed in soils sampled from environments treated with 25% aerobically digested POME while the highest (201.0±0.25 IU PNP/g/ 24h) was in mesocosms treated with 100% POME. However, in the anaerobically digested treatments, the corresponding values were 85.5±0.14 and 213.5±0.14 IU PNP /g/ 24h respectively. Thus enzyme activity was higher in the soils treated with anaerobically digested POME than the aerobically treated portions. The alkaline phosphatase activity (Table 8) were significantly ($p < 0.05$) lower than the acid phosphatase data obtained although there was no difference in activity ($p < 0.05$) between the soils receiving the aerobically digested and those treated with the anaerobically digested POME.

Table 8: Effect of different POME concentration on the soil alkaline phosphatase activity (IU PNP/g/ 24h) after several weeks

Week	POME Concentration (%)								
	Control	Aerobically Digested POME				Anaerobically Digested POME			
		25	50	75	100	25	50	75	100
1	11.8±0.03 ^{2c}	10.3±0.01 ^{3a}	13.55±0.01 ^{1a}	14.85±0.03 ^{1a}	19.25±0.01 ^{1a}	9.43±0.01 ^{4a}	10.13±0.01 ^{3a}	12.1±0.14 ^{2a}	17.95±0.03 ^{1a}
2	11.73±0.03 ^{2ab}	10.8±0.03 ^{2b}	13.95±0.01 ^{1b}	15.25±0.03 ^{1b}	21.58±0.03 ^{1b}	9.98±0.01 ^{4a}	10.0±0.03 ^{3a}	12.53±0.01 ^{2b}	20.85±0.01 ^{1b}
3	11.5±0.14 ^{2b}	12.1±0.14 ^{2c}	14.68±0.03 ^{2c}	15.98±0.01 ^{1c}	22.48±0.03 ^{1a}	10.7±0.01 ^{4c}	12.25±0.01 ^{2c}	13.58±0.01 ^{2c}	20.73±0.01 ^{1c}
4	11.78±0.01 ^{2b}	12.33±0.02 ^{2c}	14.65±0.01 ^{1c}	15.73±0.01 ^{1c}	22.63±0.01 ^{1a}	11.25±0.07 ^{4c}	12.2±0.14 ^{2c}	14.18±0.01 ^{2c}	21.98±0.01 ^{1c}
5	12.45±0.03 ^{2c}	15.13±0.01 ^{1c}	15.58±0.01 ^{1c}	16.78±0.01 ^{1a}	23.58±0.01 ^{1a}	12.53±0.01 ^{4a}	12.65±0.01 ^{2c}	14.95±0.03 ^{2a}	22.28±0.01 ^{1a}

KEY:

Alphabets in capital letters = Mean difference significant between various %concentration of POME treatment

Alphabets in small letters = Mean difference significant within a particular %concentration of POME treatment

NOTE: Same capital letters are not statistically different among treatments by the Turkey test ($p < 0.05$) and small lower cap letters are not statistically different among weeks by the Turkey test ($p < 0.05$) ± standard deviation.

Acid phosphatase acts best in acid environment while the alkaline phosphatases are mostly active in alkaline medium. One factor that influences the secretion of acid phosphatase in soils is the availability of soluble phosphate ion. In soil ecosystems, these enzymes play critical roles in the phosphorus-cycle and are connected to phosphate-stress and plant growth. During phosphorus-deficiency in the soil, acid phosphatase secretion from plants roots and micro-organisms are increased to enhance the solubilization of phosphates. Thus microbial activity has a direct influence on soil fertility and Promotes the ability of plants to cope under phosphorus limited conditions (Kai et. al., 2002; Karthikenyam et. al., 2002).

The activity of the urease enzyme decreased in the soil as the concentration of POME increased (Table 9). However within each treatment, activity increased as the weeks progressed until the 4th week after which significant decline ($p < 0.05$) in activity occurred. In the assays conducted after week 1 in soils amended with 25% aerobically digested POME, urease activity increased from 0.415 ± 0.007 to 2.984 ± 0.006 Ppm $\text{NH}_4^+ \text{-N} / \text{g} / 24\text{h}$ in week 4 before dropping to the final concentration of 1.851 ± 0.001 Ppm $\text{NH}_4^+ \text{-N} / \text{g} / 24\text{h}$ after the 5th week. With increase in POME concentrations, the activity decreased to 0.300 ± 0.003 Ppm $\text{NH}_4^+ \text{-N} / \text{g} / 24\text{h}$ when the soil was treated with 50% POME; and further down to 0.044 ± 0.001 Ppm $\text{NH}_4^+ \text{-N} / \text{g} / 24\text{h}$ at 100% POME treatment. However in relation to the respective treatments, urease activity was higher ($p < 0.05$) in soils from the rhizosphere of the mesocosms treated with aerobically digested POME than the anaerobically digested treatments. Urease is an enzyme that catalyzes the hydrolysis of urea in the soil into NH_3 and CO_2 with resultant increase in soil pH (Andrews et. al., 1989).

Table 9: Effect of different POME concentration on soil urease activity (Ppm $\text{NH}_4^+ \text{-N} / \text{g} / 24\text{h}$) after several weeks

Week	Control	POME Concentration (%)							
		Aerobically Digested POME				Anaerobically Digested POME			
		25	50	75	100	25	50	75	100
1	1.167±0.001 ^{2a}	0.415±0.007 ^{2a}	0.300±0.003 ^{2a}	0.118±0.004 ^{2a}	0.044±0.001 ^{2a}	0.311±0.000 ^{2a}	0.163±0.003 ^{2a}	0.085±0.004 ^{2a}	0.027±0.003 ^{4a}
2	1.172±0.002 ^{2a}	1.000±0.028 ^{2a}	0.578±0.00 ^{2a}	0.441±0.001 ^{2a}	0.379±0.001 ^{2a}	0.807±0.000 ^{2a}	0.418±0.005 ^{2a}	0.204±0.001 ^{2a}	0.164±0.003 ^{4a}
3	1.176±0.003 ^{2a}	2.775±0.021 ^{2a}	1.994±0.001 ^{2a}	1.801±0.001 ^{2a}	1.018±0.000 ^{2a}	1.523±0.004 ^{2a}	1.465±0.001 ^{2a}	1.335±0.001 ^{2a}	0.938±0.000 ^{4a}
4	1.228±0.001 ^{2a}	2.984±0.006 ^{2a}	2.930±0.014 ^{2a}	2.594±0.006 ^{2a}	2.437±0.004 ^{2a}	2.691±0.014 ^{2a}	2.264±0.005 ^{2a}	2.187±0.001 ^{2a}	2.149±0.001 ^{2a}
5	1.177±0.001 ^{2a}	1.851±0.001 ^{2a}	1.839±0.001 ^{2a}	1.670±0.014 ^{2a}	1.615±0.001 ^{2a}	1.814±0.001 ^{2a}	1.559±0.003 ^{2a}	1.547±0.001 ^{2a}	1.500±0.014 ^{2a}

KEY:

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This feature is vital to the regulation of nitrogen supply to plants after urea fertilization. Cookson and Lepiece (1996) reported increased urease activities in nitrogen rich soils. However, the activity of urease in soils is influenced by many factors which include cropping history, organic matter, pH, depth, soil amendments and environmental factors such as temperature (Yang et. al., 2006).

Conclusion

POME has high phenol content and is acidic in nature. It therefore must undergo some form of treatment before application as manure on land. When decomposed, POME contains rich organic materials, near alkaline pH, substantial amounts of plant nutrients and thus could serve as low cost fertilizer for crops. In the present study, the use of digested POME improved keysoil microbiological and biochemical indices but excessive application beyond plant requirement and soil absorptive capacity could still pose dangers to the soil ecosystem. It is imperative therefore to monitor nutrient release from POME in relation to crop improvement. It is also necessary that POME should be properly treated before discharge into the environment to avoid unwholesome and untoward ecological situations.

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