

## BIOREMEDIATION OF PALM OIL MILL EFFLUENT (POME) CONTAMINATED SOIL USING ORGANIC AMENDMENTS

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### Abstract

Palm Oil Mill Effluent (POME) is a high-strength organic waste that poses significant environmental risks, particularly in regions where untreated discharge into terrestrial ecosystems is common. The objective of this study is to evaluate the influence of organic waste on bioremediation of POME. The study was a factorial experiment laid out in a completely randomized design, consisting of four treatments and two control (soil only and soil amended with POME) with six replications. Standard dilution methods were used for the isolation of bacteria and fungi. The estimation of colony forming unit per mL (cfu g<sup>-1</sup>) was assessed using 10-fold serial dilution method. The physiochemical parameters analyzed were pH, chemical oxygen demand, biochemical oxygen demand and total organic content using standard laboratory procedures. The results showed that the pH values of POME treated with organic amendment (6.2) was significantly higher at P<0.05 than that of unamended POME (5.1). The organic carbon in POME amended with organic waste (1.6 %) was statistically higher at P<0.05 than that of unamended POME (0.4 %). The BOD of the unamended POME (147.61 mg/kg) was statistically higher at P<0.05 than combined organic amendment (123.59 mg/kg). The results indicate that combining organic treatments is more effective than single dosage in bioremediation of POME contamination. This study provides critical localized data on POME-induced soil alterations, informing future remediation strategies aimed at sustainable agro-industrial practices. Effective management of POME discharge is essential to safeguard soil health, agricultural productivity, and environmental quality in palm oil-producing regions.

**KEYWORDS:** Bioremediation, Organic amendment, Palm oil mill effluent (POME), Soil contamination, Soil amendment

## Introduction

Oil palm production has been recognized for its contribution towards the economic growth and sustenance of most palm oil producing communities in Nigeria. Contrary to its economic benefits, it has also contributed to environmental pollution due to the production of huge quantities of by-products from the extraction process. Apart from palm oil and palm kernel, the processing of oil palm also produces copious amounts of waste commonly referred to as palm oil mill effluent (POME).

It has been observed that most of the POME produced by small-scale traditional operators in Nigeria undergo little or no treatment and is usually discharged into the surrounding environment (Okwute, 2007). POME is often released untreated, leading to environmental issues such as soil acidification, nutrient imbalance, and potential pollution of water bodies (Ahmad *et al.*, 2010). Hence, it is important to treat the effluent to its best degree before discharged to the environment to avoid leaving impact to human health and pollution (Abubakar *et al.*, 2021; Kamyab *et al.*, 2018).

POME is a brown slurry of organic solids (4-5%), residual oil (0.5-1.0%) and water (95%) which is generated mainly from palm oil extraction, washing and cleaning processes in the mill (Agamuthu, 1995). POME is characterized with high organic content, high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), (Maheswaran and Singam, 1977). It is known to cause environmental adverse effect such as eutrophication and freshwater pollution. The effect of release of untreated POME into the environment has been reported, leading to loss of biodiversity and soil fertility. Raw POME or partially treated POME usually contains extremely high content of degradable organic matter which is due to the

presence of unrecovered palm oil (Ahmed *et al.*, 2003)

Degradation of pollutants in the natural environment takes place slowly by activities of microorganisms. This will result to harmful effect in the ecosystem before such environment recovers. In order to hasten the rate of recovery of polluted environments, bioremediation technologies are applied. Bioremediation is the use of biological processes and agent especially microorganisms their enzymes and green plants to degrade the environmental contaminants into less toxic forms, thereby returning the natural environment altered by pollutants to its original condition (Vidali, 2001; Khan, 2011).

In recent years, the application of organic amendments in agriculture has gained global attention as a sustainable approach to enhance soil health and plant productivity, especially in regions where synthetic fertilizers are either costly or environmentally detrimental (Osman *et al.*, 2019). Organic amendments like cow dung, poultry droppings, and pig dung are rich in essential nutrients, promoting soil microbial activity and plant nutrient uptake (Sulaiman *et al.*, 2019).

Despite the recognized potential of POME and organic amendments in enhancing soil fertility, there is still dearth of information on various microbial activities for biodegradation of POME. Therefore, it is imperative to investigate the various microorganisms responsible for the degradation at different stages as this will help in optimizing the biodegradation processes. This study aimed to evaluate efficacy of organic wastes in bioremediation of POME.

## Materials and methods

### Study Area

The study was conducted between the month of May and August, 2024; at the Screen House located behind the Department of Crop and Soil Science, University Park, University of Port Harcourt, Rivers State, Nigeria. Port Harcourt is found in the subequatorial region of Nigeria. Port Harcourt lies between  $4^{\circ}07'$  and  $5^{\circ}5'$ N and longitude  $60^{\circ}56'04''$  and  $7^{\circ}3'20''$ E on an elevation of 18 m above sea level. The mean annual rainfall ranges from about 3000 - 4500mm with a bimodal pattern, starting in March and ending in November with peaks in June and September and short period of dry spell in August usually known as August break (Numbere *et al.*, 2016).

### Sample Collection

Palm Oil Mill Effluent (POME) polluted soil sample was collected from a polluted site around Palm Oil Mill located at Omuahunwo Aluu Community in Ikwerre Local Government Area of Rivers State, South-South, Nigeria. Bulked composite samples were collected using soil auger. The samples were pooled together for homogeneity into sterile black polyethylene bag and transported to the laboratory and stored in the refrigerator at  $4^{\circ}\text{C}$ . Cow dung was obtained from an abattoir in Aluu community, Ikwerre Local Government Area of Rivers State, Nigeria. Pig dung and poultry droppings from University of Port Harcourt Demonstration farm respectively. Each of the organic waste aforementioned was collected into sterile polythene bag. It was composted for two weeks to reduce its pathogenic effect on the environment (Sample *et al.*, 2001).

### Experimental Design

The study was a factorial experiment laid out in a completely randomized design (CRD), consisting of four (4) treatments plus two controls (pot with bare soil and a pot with soil

mixed with POME) with six (6) replications. The first factor comprises of three (3) types of organic amendments (0.33 kg of each organic manure) namely, (i) poultry droppings (ii) pig manure (iii) cow dung at a certain application rate of 1kg per pot. The second factor was POME at a certain application rate of 100 ml per pot. A total of 10 kg of composite soil was collected from a mini farm located behind the Department of Crop and Soil Science, University of Port Harcourt and carefully filled into 36 planting pots respectively using a hand shovel.

### Reagents and Media

All reagents employed in this study were of analytical grade and were products of Sigma Chemical Company, St. Louis, Missouri, USA and BDH Chemical, Ltd, Poole, England. All microbiological media used were products of Oxoid and Difco Laboratories England (Nutrient Agar (NA), Patato dextrose agar (PDA) and MacConkey's agar).

### Enumeration of Bacteria and Fungi Populations

The Total Culturable Heterotrophic Bacteria Counts (THBC) of the POME contaminated soil and amended soil samples were carried out using spread plate method on nutrient agar (NA) (oxoid) (APHA, 1998). Serial ten-fold dilutions were prepared with normal saline. One gram of soil sample was weighed into test tube containing 9ml normal saline. This was repeated up to  $10^{-5}$ . Aliquots (0.1ml) of  $10^{-4}$  –  $10^{-5}$  dilutions were inoculated onto NA plates in triplicates. The plates were incubated at  $37^{\circ}\text{C}$  for 24h. The same procedure was used for total fungal (TF) counts, inoculating 1ml of  $10^{-4}$  –  $10^{-5}$  dilutions onto Potato Dextrose Agar (PDA) plates incorporated with lactic acid to inhibit the growth of bacteria. The plates were incubated at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 3-5 days. Plates were enumerated after incubation periods and

expressed as colony forming units per gram (cfu/g).

### **Isolation and Identification of Bacterial and Fungal Isolates**

Culturable bacterial isolates from NA plates were purified by sub-culturing onto NA plates and incubated at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 24 hours. Discrete colonies were further subcultured onto NA slants in Bijou bottles and incubated at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 24 hours. The NA slants were stored in the refrigerator at  $4^{\circ}\text{C}$  as pure stock cultures. The pure bacterial isolates were identified based on colonial and cell morphology as well as biochemical characteristics with reference to Bergey and Holt, (1994); Cheesbrough, (2006). Moulds were identified based on macroscopic and microscopic appearances which include, pigmentation, aerial and substrate hyphae. Isolates were placed on clean and grease free slides with drop of lactophenol and covered with coverslips. The isolates were identified using the scheme of Domsch and Gams (1970) and David et al. (2007).

### **Determination of Physicochemical Parameters**

The physicochemical parameters of the POME contaminated and amended soil samples analysed were pH, Chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total organic content (TOC). All the parameters were determined using standard laboratory procedures adopted from ASTM (2003) and Stewart et al. (1974) the pH was determined using Hach pH Meter (Model EC10).

### **Duration of the Study**

This study lasted for three (3) months.

### **Statistical Analysis**

Data collected from the various parameters were subjected to analysis of variance using SPSS (Version 19.0) (SPSS, 2023) computer

package to compare treatment values. Mean difference was separated using the least significant difference at 5 % level of probability.

## **Results and discussion**

### **Soil Reaction**

It was observed that the pH values of POME treated with organic amendment with range  $5.7 \pm 4.32$  –  $6.2 \pm 3.13$ ; were significantly higher at  $P < 0.05$  than that of unamended POME (5.1) as shown in Table 1. The pH of POME treated with organic amendments were not statistically different from each other. The increase in pH values from  $5.1 \pm 2.03$  to  $6.2 \pm 3.10$  indicates reduction in soil acidity. The high acidic level (pH  $5.10 \pm 2.03$ ) of the POME contaminated soil could be attributed to the acidic nature of POME as observed by Bek-Nielsen et al., (1999). The acidity is as a result of the accumulation of organic acids in the sample due to fermentation process by indigenous microorganisms (Parveen et al., 2010; Ibe et al., 2014). The pH value was fairly below the recommended value by the Federal Environmental Protection Agency (FEPA) of Nigeria (1991) effluent limitation guideline of pH 6 – 9.

### **Total Organic Carbon (TOC)**

Generally, OC values of POME treated with amended organic amended ranged between  $0.8 \pm 3.14$  % –  $1.6 \pm 4.32$  % were statistically higher than that of unamended POME (0.4 %) as shown in Table 1. However, POME treated with combined cow dung, pig dung and poultry droppings recorded the highest OC value ( $1.6 \pm 4.32$  %) which is statistically higher than the rest values of OC while the

least was that of unamended soil which is statistically lowest than other OC values. The value of TOC in POME amended samples were significantly higher than that of unamended POME samples. The amendment introduces additional carbon-rich materials such as carbohydrates, proteins, lipids, and lignocellulosic compounds. These compounds increase the overall concentration of organic matter in the effluent. This observation is in agreement with the findings of Chukwuma *et al.* (2018) who reported that organic amendments like poultry droppings and cow manure enhanced microbial biomass and activity, leading to improved plant growth. The increase in microbial activity supported nutrient cycling, which in turn benefited crop performance (Chukwuma *et al.*, 2018).

### Chemical oxygen demand (COD)

The COD of unamended POME ( $196.28 \pm 2.00$  mg/kg) was statistically higher than those of POME amended values ranged between  $96.19 \pm 1.34$  mg/kg –  $104.63 \pm 2.11$  mg/kg as shown in Table 1. However, the POME amended with combined poultry droppings, cow dung and pig dung showed the lowest value of COD ( $96.19 \pm 1.34$  mg/kg) which is significantly lowest at  $P < 0.05$  among other treatments.

### Biochemical oxygen demand (BOD)

The BOD of the unamended POME ( $147.61 \pm 5.09$  mg/kg) was statistically higher than those of POME treated with organic amendments as shown in Table 1. However, the POME amended with combined poultry droppings, cow dung and pig dung showed the lowest value of BOD ( $123.59 \pm 4.27$

mg/kg) which is significantly lowest at  $P > 0.05$  among other treatments.

There were significant changes in physicochemical characteristics of POME contaminated soil and soil treated with organic amendments dung during the bioremediation period under study. The results showed that there were reductions in COD and BOD of the POME contaminated soil amended with organic amendments compared to the unamended counterparts. The values of other physicochemical parameters of the POME contaminated soil, COD and BOD showed high values when compared to the POME contaminated soil amended with cow dung during the study period. These may be due to the constituents of the POME which include cellulose fruit debris, degradable organic matter and unrecovered palm oil (Ahmed *et al.*, 2003). The reductions in the physicochemical parameters in the amended POME contaminated soil with organic amendments was as a result of the high microbial load in the cow dung that enhanced the biodegradation of the organic pollutants in the POME contaminated soil (Owute and Isu, 2007; Owkwute and Ijah, 2014).

### Total Fungal Count

POME amended with combined cow dung, poultry droppings and cow dung recorded the highest fungal count ( $9.0 \times 10^3 \pm 4.32$ ) which is statistically the same with that of POME amended with Poultry droppings ( $8.9 \times 10^3 \pm 4.12$ ) but significantly higher than the rest of treatments (Table 2). Next in line was POME amended with cow dung ( $7.5 \times 10^3$  cfu g<sup>-1</sup>) which is statistically with soil without POME contamination. ( $6.0 \times 10^3$  cfu g<sup>-1</sup>). On

the other hand, the least fungal count among the treatment were Unamended POME ( $5.4 \times 10^2 \pm 2.08$  cfu g<sup>-1</sup>) and POME amended with both Pig Dung + POME ( $4.7 \times 10^3 \pm 2.12$  cfu g<sup>-1</sup>).

### Total Bacterial Count

POME amended with Poultry droppings recorded the highest bacterial count ( $8.7 \times 10^7 \pm 4.12$  cfu g<sup>-1</sup>) which is statistically the same with that of POME amended with cow dung ( $8.5 \times 10^7 \pm 4.32$  cfu g<sup>-1</sup>) but significantly higher than the rest of treatments (Table 2). Next in line is the POME amended with Pig dung ( $7.5 \times 10^6 \pm 3.23$  cfu g<sup>-1</sup>) and amendment with the combined cow dung, poultry droppings and cow dung. ( $7.5 \times 10^6 \pm 3.12$  cfu g<sup>-1</sup>). However, the least bacterial count was recorded in Untreated POME and it was not statistically different from that of Soil alone without POME ( $4.5 \times 10^6 \pm 2.73$  cfu g<sup>-1</sup>).

The microbial populations of the untreated POME contaminated soil showed statistically lower total heterotrophic bacterial (THB) count ( $3.5 \times 10^5 \pm 2.03^c$  cfu/g) and total fungal (TF) ( $5.4 \times 10^2 \pm 2.08^c$  cfu/g) as shown in Table 2. The lower bacterial counts recorded in the unamended POME contaminated soil may be attributed to the high acidity and oily content as only microorganism with the competent enzyme systems to proliferate can thrive in it. It was observed that the Bacteria in unamended POME were greater than those of fungi. This observation is in tandem with the findings of Benneth and Fasion (1997) who attributed the dominance of bacteria degraders to the fact that fungi are more proficient at co-metabolism and bioaccumulation than at using pollutants as sole carbon source, hence the higher THB

counts than TF counts throughout the period of bioremediation. This study observes positive effects of organic amendment on bioremediation of POME contaminated soil as typified in COD of soil without organic amendment ( $196.28 \pm 2.00$ ) while that with poultry droppings cow dung and pig dung gave  $101.25 \pm 3.13$ ,  $103.50 \pm 3.09$ ,  $104.63 \pm 2.11$  respectively. This observation is in agreement with the findings of Okwute and Ijah, 2014; Obire et al., 2008 who reported a positive effect of organic nutrient supplement on bioremediation of POME contaminated soil.

### Distribution of isolated Bacteria

The distribution of isolated bacteria across the various treatment is represented in Table 3. The following bacterial isolates were present in all treatments: *Pseudomona aeruginosa*, *Bacillu. spp* and *Proteus vulgaris*. It was observed that *Staphylococcus aureus* was present in all treatment except in POME amended with Poultry droppings. Similarly, *Micrococcus. roseus* was isolated in all treatments except in POME amended with Pig dung. On the other hand, *Escherichia. coli* was isolated form all the treatments except on uncontaminated soil and soil with untreated POME.

### Distribution of isolated Fungi

The distribution of isolated fungi across the various treatment is represented in Table 4 below. The following fungal isolates were present in all treatments: *Aspergillus. niger*, *Penicillium. Verrucosum*, *Candida. albicans* and *Rhodotorula. rubra*. it was observed that *Mucor mucedo* was isolated only in uncontaminated soil. *Fusarium. spp* was isolated only in uncontaminated soil,

POME amended with cow dung and POME amended with combined poultry dropping, cow dung and Pig dung. *Trichophyton. spp* was isolated in all treatment except in unamended POME. *Rhizopus. oryzae* was isolated only in uncontaminated soil and POME amended with combined cow dung, poultry droppings and pig dung. *Paecilomyces. lilacinum* was isolated in all treatment except in uncontaminated soil and unamended POME. *Saccharomyces. cerevisiae* was isolated only in uncontaminated soil, unamended POME and POME amended with combined cow dung, poultry droppings and pig dung. *Rhodotorula. rubra* was isolated only in POME amended with combined cow dung, poultry dropping and pig dung.

The results of isolation and identification of bacteria from the amended soil samples to the generic level revealed the following; *Pseudomonas. aeruginosa*, *Bacillu. spp*, *Staphylococcus. aereus*, *Escherichia. coli* and *Proteus. vulgaris* while the fungal genera included *Rhodotorula. rubra*, *Saccharomyces. cerevisiae*, *Candida. albicans*, *Paecilomyces. lilacinum*, *Penicillium. verrucosum*, *Fusarium. spp*, *Trichophyton. spp*, *Aspergillus. niger*. Similar organisms have been identified in previous studies on bioremediation of POME polluted soil and crude oil polluted soil using microorganism found in organic wastes

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(Obire et al., 2008; Okwute and Ijah, 2014). The present study shows that these isolates (bacteria and fungi) have the degradative ability to degrade the organic pollutants in the POME polluted soil sample. It is noteworthy that the levels of microorganisms vary amongst the treatment mainly due their varying nutrient (carbon) requirement and preference.

## Conclusion

In conclusion, the findings of this study, suggests that the application of cow dung increased microbial populations in the POME contaminated soil, increased TOC and pH values, thereby reducing the acidity and reduction in BOD, COD and TOC of the POME contaminated soil. The bacterial and fungi genera isolated have the potential to degrade the organic pollutants in the POME contaminated soil and can be applied in the ecofriendly technology of clean-up of chemical or hydrocarbon contaminated sites.

The cow dung, poultry droppings and pig dung could be applied independently in bioremediation of POME-contaminated soil. However, combining them together as a combo organic amendment is more effective than single treatment in degrading of pollutants in POME-contaminated soil as ecofriendly bioremediation technology.

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**Table 1: Physicochemical characteristics of POME treated with organic amendments**

Parameter	Unit	S+ P	S + C + P	S+ PO + P	S+ PI+ P	S+ P + PI + PO + C
pH	-	5.1±2.03 <sup>b</sup>	6.2±3.10 <sup>a</sup>	6.0±3.87 <sup>a</sup>	6.2±3.13 <sup>a</sup>	5.7±4.32 <sup>a</sup>
OC	%	0.4±2.01 <sup>c</sup>	1.1±3.64 <sup>b</sup>	1.0±3.25 <sup>b</sup>	0.8±3.14 <sup>b</sup>	1.6±4.32 <sup>a</sup>
COD	mg/kg	196.28±2.00 <sup>a</sup>	103.50±3.09 <sup>b</sup>	101.25±3.13 <sup>b</sup>	104.63±2.11 <sup>b</sup>	96.19±1.34 <sup>c</sup>
BOD	mg/kg	147.61±5.09 <sup>a</sup>	133.30±5.19 <sup>b</sup>	130.10±2.89 <sup>bc</sup>	134.43±4.19 <sup>b</sup>	123.59±4.27 <sup>c</sup>

P = pome, PI = pig dung, PO = poultry droppings, C = cow dung, S = Soil, A = alone OC = organic carbon, TN = Total Nitrogen, Ex. = exchangeable, Av.P = Available Phosphorus. Means followed by the same alphabets within column were not significantly different at p <0.05

**Table 2: Microbial counts of soil fortified with various organic amendments after harvest**

Treatments	TBC		TFC	
			(cfu g <sup>-1</sup> )	
Soil Alone		$4.5 \times 10^6 \pm 2.73^c$		$6.0 \times 10^3 \pm 3.02^b$
Soil + Pome		$3.5 \times 10^5 \pm 2.03^c$		$5.4 \times 10^2 \pm 2.08^c$
Soil + Cow Dung+ Pome		$8.5 \times 10^7 \pm 4.32^a$		$7.5 \times 10^3 \pm 3.12^b$
Soil + Poultry droppings + Pome		$8.7 \times 10^7 \pm 4.12^a$		$8.9 \times 10^3 \pm 4.12^a$
Soil + Pig Dung +Pome		$7.5 \times 10^6 \pm 3.23^b$		$4.7 \times 10^3 \pm 2.12^c$
Soil + Pig Dung +Pome+ Poultry droppings+ Cow Dung		$7.5 \times 10^6 \pm 3.12^b$		$9.0 \times 10^3 \pm 4.32^a$

CFU g<sup>-1</sup> = Colony forming unit per gram, TFC = Total Fungi count, TBC = Total Bacterial count. Means of the same alphabet are not significantly different at P ≥ 0.05.

**Table 3: Distribution of bacteria across treatments**

Isolate	SA	S + P	S + C + P	S + PO + P	S + PI+ P	S + P + PI + PO + C
<i>Pseudomonas. Aeruginosa</i>	+	+	+	+	+	+
<i>Bacillu. Spp</i>	+	+	+	+	+	+
<i>Staphylococcus. Aereus</i>	+	+	+	-	+	+
<i>Escherichia. Coli</i>	-	-	+	+	+	+
<i>Proteus. Vulgaris</i>	+	+	+	+	+	+
<i>Micrococcus. Roseus</i>	+	+	+	+	-	+

+ = Isolated, - = Not isolated, P = pome, PI = pig dung, PO = poultry droppings, C = cow dung, S = Soil, A = alone

**Table 4: Distribution of fungi across treatments**

Isolate	SA	S + P	S + C + P	S + PO + P	S + PI+ P	S + P + PI + PO + C
<i>Aspergillus. niger</i>	+	+	+	+	+	+
<i>Mucor. Mucedo</i>	+	-	-	-	-	-
<i>Penicillium. Verrucosum</i>	+	+	+	+	+	+
<i>Fusarium. Spp</i>	+	-	+	-	-	+
<i>Trichophyton. spp.</i>	+	-	+	+	+	+
<i>Rhizopus. Oryzae</i>	+	-	-	-	-	+
<i>Paecilomyces. lilacinum</i>	-	-	+	+	+	+
<i>Candida. Albicans</i>	+	+	+	+	+	+
<i>Sacchoromyces. Cerevisiae</i>	+	+	-	-	-	+
<i>Torulopsis. Candida</i>	-	-	-	-	-	+
<i>Rhodotorula. Rubra</i>	+	+	+	+	+	+

+ = Isolated, - = Not isolated, P = pome, PI = pig dung, PO = poultry droppings, C = cow dung, S = Soil, A = alone