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# Assessing the influence of palm oil mill effluent on soil microbial communities and associated physicochemical parameters

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**Abstract**
This research investigates how Palm Oil Mill Effluent (POME) affects the microbial communities in soil and how it changes the related physicochemical properties. Soil samples were collected from three palm oil mill vicinity at Omerelu, in Ikwerre Local Government of Rivers State, Nigeria. The samples were aseptically collected and transported to microbiology laboratory for microbiological and physicochemical analyses using standard procedures. Results obtained showed that the total heterotrophic bacteria count ranged from 7.6 x $10^5$ CFU/g to 9.2 x $10^5$ CFU/g. Fungi count ranged between 3.0 x $10^4$ CFU/g to 4.3 x $10^4$ CFU/g. The palm oil utilizing bacteria count ranged from 1.7 x $10^3$ CFU/g to 3.6 x $10^3$ CFU/g. Palm oil utilizing fungi ranged from 1.0 x $10^2$ CFU/g to 3.1 x $10^2$ CFU/g. The following bacteria genera were isolated from the soil samples, *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Micrococcus*, *Enterococcus*, *Proteus*, *Klebsiella*, *Staphylococcus*, *Corynebacterium* and *Serratia*. The highest frequency occurrence was observed in *Staphylococcus* (18%). Fungal genera isolated were *Aspergillus*, *Candida*, *Mucor*, *Rhizopus*, *Fusarium*, *Penicillium*, *Cladosporium* and *Saccharomyces*, with *Aspergillus* having the highest occurrence of 24.3%. The results of the physicochemical analysis showed that the pH value of the soil samples ranged between 5.92 to 6.67, the electrical conductivity ranged from 40.53 to 400.62 us/cm. For the salinity, nitrate and phosphate, the values ranged from 3.518 mg/g to 7.978 mg/g, 0.04 mg/g to 0.31 mg/g and 1.04 mg/g to 1.49 mg/g, respectively. Calcium and magnesium ranged from 27.47 mg/g to 43.07 mg/g and 32.63 mg/g to 54.12 mg/g, respectively. Total organic matter (Tom) ranged from 7.13% to 26.36%. Potassium and lead recorded least value of 26.74 mg/g to 48.93 mg/g and 0.913 mg/g to 5.267 mg/g, respectively. The oil and grease ranged from 52 to 3500 mg/g. These results demonstrate the diverse microbial presence in the soil affected by POME and illustrate significant variations in the soil's physicochemical attributes across the studied locations.

**Keywords:** Palm oil mill effluent, soil, total heterotrophic bacteria, fungi, physicochemical

## Introduction
Palm oil mill effluent (POME) is a byproduct generated during the extraction of crude palm oil from the oil palm fruit. This effluent is a complex mixture of water, oil, and various organic and inorganic compounds, making it a significant environmental concern in regions where palm oil is produced (Sethupathi, 2004) [9]. The palm oil milling process results in the generation of large volumes of POME, and without proper management and treatment, it can have detrimental effects on both the environment and public health (Ujang *et al.,* 2010) [10]. Countries with significant palm oil production, including Nigeria, often have environmental regulations governing the discharge of POME. Compliance with these regulations is crucial to mitigating the environmental impact of palm oil milling activities (Poku, 2002) [7]. The discharge of untreated or improperly treated Palm Oil Mill Effluent (POME) can have significant negative effects on soil quality and fertility. The impact depends on various factors such as the composition of the effluent, soil characteristics, and management practices. POME contains high levels of organic matter, nitrogen, and phosphorus. While these nutrients are essential for plant growth, excessive application without proper management can lead to nutrient imbalances in the soil. This can affect the availability of other essential nutrients and disrupt the natural nutrient cycling processes (Nnorum, 2012) [2]. POME may have acidic components, and if applied in large quantities, it can contribute to soil acidification. Acidic soils can negatively impact the availability of certain nutrients and affect the growth of plants. Some components of POME, such as heavy metals and other contaminants, may have toxic effects on plants and soil organisms if present in high

concentrations. This can lead to reduced plant growth and negatively impact soil microbial communities. The organic matter in POME can influence soil structure. While organic matter is generally beneficial for soil structure, excessive amounts can lead to compaction and reduced soil porosity, affecting water infiltration and root growth (Nwaugo *et al*., 2008) [3]. If POME is not properly managed and is allowed to run off into water bodies, it can contaminate surface water and, in turn, affect soil quality. The nutrients and pollutants in POME can leach into the soil, potentially causing downstream environmental problems. The improper disposal of POME on sloping terrain or without proper containment measures can lead to soil erosion. Erosion not only results in the loss of fertile topsoil but can also contribute to sedimentation in nearby water bodies (Nwoko *et al*., 2010) [4]. POME contains organic compounds that can either stimulate or inhibit microbial activity in the soil, depending on the concentration and composition (Salihu and Alam, 2012) [8]. High levels of organic matter may lead to increased microbial activity, but the presence of certain compounds in POME may have adverse effects on soil microorganisms. To mitigate these negative effects, it is essential to treat POME before its application to soil or its discharge into the environment. Treatment methods such as anaerobic digestion, aerobic treatment, and ponding systems can help break down organic matter and reduce the concentrations of harmful components in POME (Alrawi *et al*., 2013) [1]. Regulatory measures and adherence to sustainable practices, such as those outlined by organizations like the Roundtable on Sustainable Palm Oil (RSPO), are also crucial for minimizing the environmental impact of palm oil production, including the management of POME. Sustainable practices aim to ensure that the palm oil industry operates in an environmentally responsible manner, considering the long-term health of ecosystems and communities. This study therefore investigated the impact of palm oil mill effluent on soil microorganisms and its physicochemical constituents.

## Materials and Methods

### Study Area

The study was conducted in Omerelu, Ikwerre Local Government Area of Rivers State. The geographical location coordinate of the L.G.A is Latitude: 5º3'19"N, 6º55'21"E. The study area was chosen for the research because of palm oil mill business that is practiced in the area.

### Sample Collection

Soil samples were obtained in three different palm oil mills in Omerelu community, in Ikwerre local government area of rivers state, Nigeria. The palm oil mill effluent (POME) soil samples were collected at a depth of 0-20cm with sterile hand auger. It was carefully coded as sample A, B and C. the control soil coded D were collected in biological garden, Rivers State University. The well labelled soil samples were carefully placed in sterile zip-locked polythene bags and carefully packed in an ice packed cooler and immediately transported to the Microbiology Laboratory, Department of Microbiology, Rivers State University for microbiological analysis.

### Microbiological Analysis of on Soil Sample

### Serial Dilution

The ten-fold serial dilution was adopted. In this method, one gram (1 g) of the soil samples were weighed out and transferred into test tubes containing sterile 9 ml diluent (Normal saline). The soil samples were well shaken to homogenised suspension and thereafter, ten-fold (10-fold) serial dilution was made by aseptically transferring 1 ml of the homogenised suspension into the next sterile test tube ($10^{-2}$) containing 9 ml of sterile normal saline. It was serially diluted tube by tube until $10^{-6}$ dilution factor.

### Isolation and Enumeration of Total Heterotrophic Bacteria (THB)

The counts of total heterotrophic bacteria in the soil samples were determined by spread plate technique using a sterile pipette, 0.1 ml aliquots of the dilutions of $10^{-5}$ and $10^{-6}$ in duplicate were plated on already prepared sterile nutrient agar plates. It was spread evenly with flamed sterilized glass spreader (bent glass rod). The inoculated plates were incubated at 37 °C for 24 hours. After incubation, plates that contained 30-300 colony forming units (CFU) were counted with the aid of colony counter. Viable numbers of bacterial colonies on each plate were enumerated and expressed or recorded as colony forming units per gram (CFU/g) of the soil samples.

### Isolation and Enumeration of Total Heterotrophic Fungi (THF)

The total heterotrophic fungi (THF) counts were determined by spread plate method. An aliquot of 0.1 ml was plated in duplicate onto the already prepared PDA used for the plating. It was evenly spread using bent glass rod and incubated in an inverted position at room temperature (28 °C to 30 °C) for 3-5 days. After incubation period, fungi counts were reported as colony forming unit per gram (CFU/g).

### Isolation and Enumeration of Palm Oil-Utilizing Bacteria and Fungi (POUB and POUF)

The counts of palm oil utilizing bacteria (POUB) and palm oil utilizing fungi (POUF) in the POME soil samples were determined by plating 0.1 ml aliquots from $10^{-2}$ and $10^{-3}$ dilutions into plates of already prepared mineral salt medium (MSM) for bacteria and fungi. It was spread evenly using glass spreader. A sterile filter paper (Whatman No1) saturated with sterile palm oil was aseptically placed on the lids, that is, inside the inverted petri dishes, closed and incubated at 28 °C for 5-7 days. After the incubation period, colonies that developed on the plates were counted and recorded as counts for palm oil utilizing bacteria and fungi (POUB and POUF) and expressed as colony forming unit per gram (CFU/g) of the soil samples.

### Sub-culture/Purification of Microbial Isolates

Distinct representative of bacterial and fungal colonies was purified by repeatedly sub-cultured/transferred aseptically onto freshly prepared NA and PDA plates by the streak plate technique and incubated at 37 °C for 24 hours (Bacteria) and 72 hours (Fungi) for purification of bacterial and fungal isolates.

### Identification and Characterization of bacterial isolates

Identification of the bacterial isolates followed Bergey's manual of determinative bacteriology based on their microscopic examination, cellular morphology, colonial morphology, Gram staining and biochemical tests.

**Identification and characterization of fungal isolates**

Pure cultures obtained after 3-5 days of incubation were subjected to characterization and identification using macroscopy and microscopy methods. The morphology of the fungal growth on plates was studied including their colours. Small portions of the fungal mycelium from the plate were teased and mounted in a drop of lactophenol cotton blue stain on a clean grease-free slide using inoculating needle, and it was carefully mount/ covered with a cover slip with the help of a sterile forcep without trapping air bubbles and observed under low (x10) and high (x40) power objective lens of the microscope.

**Physiochemical Analysis of the Soil Samples**

The physiochemical analysis of the soil samples was performed using standard procedures as described by APHA, (2005) [12].

**Results**

The total heterotrophic bacteria count recorded lowest count of 7.6 x $10^5$ CFU/g in sample B and highest of 9.2 x $10^5$ CFU/g in sample D. Fungi count was lowest (3.0 x $10^4$ CFU/g) in sample A, while sample D recorded the highest count of 4.3 x $10^4$ CFU/g. The palm oil utilizing bacteria count recorded lowest count of 1.7 x $10^3$ CFU/g in sample D and highest count of 3.6 x $10^3$ CFU/g in sample C. Palm oil utilizing fungi recorded lowest count of 1.0 x $10^2$ CFU/g in sample D, while highest count of 3.1 x $10^2$ CFU/g was recorded in sample A.

**Table 1:** Microbial Count from POME Soil Samples

| POME Soil Samples | THBC (Cfu/g) | TFC (Cfu/g) | POUB (Cfu/g) | POUF (Cfu/g) |
|---|---|---|---|---|
| Mill 1 | 8.5 x $10^5$ | 3.0 x $10^4$ | 2.5 x $10^3$ | 3.1 x $10^2$ |
| Mill 2 | 7.6 x $10^5$ | 3.7 x $10^4$ | 3.2 x $10^3$ | 2.8 x $10^2$ |
| Mill 3 | 8.0 x $10^5$ | 4.1 x $10^4$ | 3.6 x $10^3$ | 1.9 x $10^2$ |
| Control | 9.2 x $10^5$ | 4.3 x $10^4$ | 1.7 x $10^3$ | 1.0 x $10^2$ |

Key: THBC ---- Total heterotrophic Bacteria, TFC ----- Total heterotrophic Fungi, CFU/g ---- Colony forming unit per gram, POUB ---- Palm oil-utilizing Bacteria, POUF ---- Palm oil-utilizing Fungi.

The following bacteria genera were isolated from the soil samples, *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Micrococcus*, *Enterococcus*, *Proteus*, *Klebsiella*, *Staphylococcus*, *Corynebacterium* and *Serratia*. The highest frequency occurrence was observed in *Staphylococcus* (18%).

**Table 2:** Percentage Occurrence of Bacteria Isolates

| Bacterial Isolates | Frequency of occurrence | Percentage occurrence (%) |
|---|---|---|
| *Bacillus* spp | 33 | 13.5 |
| *Flavobacterium* spp | 14 | 5.7 |
| *Pseudomonas* spp | 35 | 14.3 |
| *Micrococcus* spp | 28 | 11.5 |
| *Enterococcus* spp | 18 | 7.4 |
| *Proteins* spp | 25 | 10.3 |
| *Klebsiella* spp | 12 | 4.9 |
| *Staphylococcus* spp | 44 | 18.1 |
| *Corynebacterium* spp | 9 | 3.7 |
| *Serratia* spp | 26 | 10.6 |
| Total | 244 | 100 |

Fungal genera isolated were *Aspergillus*, *Candida*, *Mucor*, *Rhizopus*, *Fusarium*, *Penicillium*, *Cladosporium* and *Saccharomyces*, with *Aspergillus* having the highest occurrence of 24.3%.

**Table 3:** Percentage Occurrence Fungi Isolate

| Fungal isolates | No. of occurrence | Percentage occurrence (%) |
|---|---|---|
| *Aspergillus* spp | 25 | 24.3 |
| *Candida* spp | 19 | 18.4 |
| *Muccor* spp | 5 | 4.9 |
| *Rhizopus* spp | 10 | 9.7 |
| *Fusarium* spp | 13 | 12.6 |
| *Penicillin* spp | 15 | 14.5 |
| *Cladosporium* spp | 9 | 8.8 |
| *Saccharomyces* spp | 7 | 6.8 |
| Total | 103 | 100 |

The results of the physicochemical analysis are recorded in Table 1. The pH value of the soil samples ranged between 5.92 to 6.67. The least value was recorded in sample D while the highest value was recorded in sample C. The electrical conductivity ranged from 40.53 (sample D) to 400.62 us/cm (sample B). For the salinity, nitrate and phosphate, the least value of 3.518 mg/g, 0.04 mg/g and 1.04 mg/g, respectively was recorded from sample D, while the highest value of 7.978 mg/g, 0.31 mg/g and 1.49 mg/g, respectively was recorded from sample B. Calcium and magnesium recorded the least value of 27.47 mg/g and 32.63 mg/g, respectively from sample D and highest value of 43.07 mg/g and 54.12 mg/g, respectively from sample C. Total organic matter (Tom) recorded least percentage of 7.13% from sample D and highest value of 26.36% from sample A. potassium and lead recorded least value of 26.74 mg/g and 0.913 mg/g from sample D, while the highest value of 48.93 mg/g of potassium was recorded from sample A, lead highest value of 5.267 mg/g was recorded from sample B. The oil and grease ranged from 52 to 3500 mg/g.

**Table 4:** Physiochemical Analysis of Samples

| Parameter | Sample A (Mill 1) | Sample B (Mill 2) | Sample C (Mill 3) | Sample D (Control) (Mill 4) |
|---|---|---|---|---|
| pH | 6.35 | 6.43 | 6.67 | 5.92 |
| EC (uS/cm) | 90.48 | 400.62 | 130.44 | 40.53 |
| Salinity (mg/g) | 6.166 | 7.978 | 5.758 | 3.518 |
| Nitrate (mg/g) | 0.13 | 0.31 | 0.23 | 0.04 |
| Phosphate (mg/g) | 1.41 | 1.34 | 1.49 | 1.04 |
| Calcium (mg/g) | 39.22 | 34.28 | 43.07 | 27.47 |
| Magnesium (mg/g) | 41.87 | 49.54 | 54.12 | 32.63 |
| TOM (%) | 26.36 | 15.46 | 10.65 | 7.13 |
| K (mg/g) | 48.93 | 38.92 | 33.27 | 26.74 |
| Pb (mg/g) | 3.420 | 5.267 | 4.081 | 0.913 |
| Oil and Grease (mg/g) | 2500 | 1500 | 3500 | 52 |

## Discussion

The variation in THBC across samples suggests that POME application influences the abundance of heterotrophic bacteria in the soil. The highest count in the control sample (Sample D) might indicate that factors unrelated to POME, such as organic matter inputs or other environmental conditions, contribute to increased bacterial populations. In line with the findings from this work, Wokem and Akpila (2021) [6]. Revealed that the lower counts recorded in the POME contaminated soil may be attributed to its acidic and oily content as only microorganisms with the competent enzyme systems to proliferate can be found in it. The lowest count in Sample B indicates a potential influence of POME on reducing bacterial abundance in that specific location. The range of fungal counts suggests variability in fungal populations across samples, with the lowest count in Sample A and the highest in the control sample (Sample D). POME may not have a pronounced effect on overall fungal abundance, but the observed fluctuations may reflect differences in nutrient availability or other environmental factors associated with POME application. This increase in fungi population maybe attributed to the acidity nature of the effluents because fungi strive best in acidic environments than bacteria which can only survive in a neutral to alkaline pH environment. The counts of bacteria capable of utilizing palm oil compounds show that POME introduces specific microbial populations to the soil. The highest count in Sample C indicates an enrichment of bacteria adapted to palm oil utilization, potentially due to exposure to POME in that location. The lowest count in the control sample (Sample D) suggests that these specific bacteria may be more responsive to POME inputs. Like palm oil utilizing bacteria, the counts of fungi capable of utilizing palm oil suggest a selective influence of POME on fungal communities. The lowest count in the control sample (Sample D) and the highest in Sample A indicate differential responses of palm oil utilizing fungi to POME exposure. This may be influenced by the composition of palm oil residues and their impact on fungal communities. Comparing microbial counts across samples highlights spatial variations in microbial responses to POME. The control sample (Sample D) consistently exhibits higher counts in THBC and Fungi, suggesting that POME application may not drastically alter overall microbial abundance in the short term. However, the amount of palm oil utilizing bacteria and fungi in other samples indicate specific responses to POME exposure. The high counts of palm oil utilizing bacteria obtained in this study was like that of and Nwaugo *et al*. (2008) [3], suggesting that the pollutant may have provided a good environment for them to flourish.

The diversity of isolated genera suggests a complex microbial community in the soil, contributing to various ecological processes. The dominance of Staphylococcus at 18% may indicate specific environmental conditions favoring the proliferation of this genus. Previous study (Osaro, 2002) [5] found that the genera *Pseudomonas* sp, *Bacillus* sp, *Penicillium* sp and *Aspergillus* sp were predominant. These microorganisms including others listed in Tables 2 and 3 were isolated in the study. The diversity of fungal genera suggests a complex fungal community in the soil, contributing to decomposition, nutrient cycling, and other ecological processes. The highest occurrence of Aspergillus indicates its prevalence in the sampled environment, which may have implications for the soil's fungal ecology and functions. Aspergillus species are known for their adaptability and ability to produce a variety of enzymes, contributing to organic matter breakdown. The ability of these microorganisms to survive in POME contaminated soil may be associated with their ability to secrete extracellular enzymes which degrade free fatty acids in POME for their carbon and energy source (Yoochatchaval, 2011) [11]. The high nutrient content of POME may also stimulate their growth and other cellular components synthesized from the waste.

The pH values ranging from 5.92 to 6.67 indicate a slightly acidic to neutral soil pH. Sample D with the lowest pH value may suggest a more acidic condition, while sample C with the highest pH may indicate a more neutral environment. Soil pH is essential for nutrient availability and microbial activity. The acidic pH in sample D may impact nutrient availability, affecting plant growth and microbial activities. The range of electrical conductivity from 40.53 to 400.62 µS/cm reflects variations in soil ion content. Higher EC in sample B suggests a higher concentration of dissolved ions, potentially influenced by anthropogenic activities or mineral content. The variations in salinity, nitrate, and phosphate levels indicate differences in nutrient and salt concentrations. Sample B exhibits the highest values, suggesting potential inputs from external sources, such as fertilizers or other agricultural practices. The levels of calcium and magnesium influence soil structure and plant nutrition. Sample D has the lowest values, indicating potential deficiencies, while sample C with the highest values suggests a more favorable environment for plant growth. The percentage of total organic matter ranges from 7.13% to 26.36%. Sample D with the lowest value may have lower organic inputs or higher decomposition rates, while sample A with the highest value suggests richer organic content, influencing soil

fertility and microbial activity. Potassium levels range from 26.74 to 48.93 mg/g, with the highest value in sample A. Lead concentrations vary from 0.913 to 5.267 mg/g, with the highest in sample B. Elevated lead levels may raise concerns about potential contamination, while higher potassium levels contribute to nutrient availability. Oil and grease levels ranging from 52 to 3500 mg/g suggest potential contamination, possibly from anthropogenic sources. Sample D with the lowest value may have lower anthropogenic influence, while sample B with the highest value indicates a higher degree of contamination. The variations in physicochemical parameters highlight the heterogeneity of the soil samples and potential influences of anthropogenic activities. Elevated electrical conductivity and salinity in sample B may indicate potential anthropogenic inputs, affecting soil quality. Varied nutrient levels, organic matter content, and contaminant concentrations can impact soil fertility, microbial communities, and overall ecosystem health.

## Conclusion

The findings suggest an impact of POME on soil microbial communities, with varying effects on bacterial and fungal populations. While POME might not dramatically alter overall microbial abundance, it does introduce specific microbial groups capable of utilizing palm oil compounds, indicating a selective influence on certain microbial populations. The physicochemical analysis indicates potential alterations in soil properties and composition following POME application. Variations in pH, nutrient content, organic matter, electrical conductivity, and heavy metal presence across samples suggest that POME can influence soil characteristics.

## Conflict of Interest

Not available

## Financial Support

Not available

## References

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