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# **Assessment of the Physicochemical and Microbiological Quality of Palm Oil Mill Effluent (POME) and Soil in Aluu, Rivers State, Nigeria**

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## *Author's contribution*

*This work was carried out by the author. Author JOW designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author JOW managed the analyses of the study, literature searches, read and approved the final manuscript.*

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## **ABSTRACT**

**Aim:** To assess the Physicochemical and microbiological quality of POME and soil in Aluu, Rivers State.

**Place and Duration of Study:** This study was carried out in Omuahunwo, Aluu near Choba, University of Port Harcourt, Rivers State and Department of Applied and Environmental Biology Farmland, Rivers State University, Port Harcourt in Rivers State.

**Materials and Methods:** Palm oil mll effluent (POME) samples used in the study were obtained from two local palm oil factories located at Omuahunwo in Aluu near Choba, University of Port Harcourt in Rivers State. Replicate samples were collected bimonthly from the same source. Soil samples were obtained from an uncultivated farmland about 2 km away from the factory and a control soil sample devoid of POME contamination was taken from Department of Applied and Environmental Biology Farmland, Rivers State University, Port Harcourt. Samples were obtained from soil in proximity to the two factories polluted with POME. Samples were analyzed for their physicochemical and microbiological qualities.

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**Results:** Palm oil Mills A and B had the following physicochemical parameters mean values: pH 5.48, 5.36; Conductivity 658*μ*mhos/cm, 756*μ*mhos/cm; total suspended solids 120,200 mg/l,122,000 mg/l;dissolved Oxygen 0mg/l (Palm oil mills A and B); BOD 5160 mg/l, 5200 mg/l;COD 432 mg/l, 4370 mg/l; Oil and Grease 165853 mg/l, 165900mg/l ;Phosphate 2.53 mg/l, 2.58 mg/l; Nitrate 68.83 mg/l, 68.90 mg/l; Ammonia 0 mg/l (for Palm Oil Mills A and B). Mean population of 8.25 x 10<sup>3</sup> cfu/ml,  $3.\overline{6}$  x 10<sup>3</sup> cfu/ml,  $3.2$  x 10<sup>2</sup> cfu/ml and 12 MPN Index/100ml were recorded for total heterotrophic bacteria, filamentous Fungi, yeast and total coliforms respectively for the effluent samples from Palm Oil Mill A. Palm Oil Mill B had the following counts for total culturable heterotrophic bacteria, filamentous Fungi, yeast and total coliforms:  $6.1 \times 10^3$  cfu/ml  $2.5 \times 10^3$  cfu/ml,  $2.2 \times 10^2$  cfu/ml and 8 MPN Index/100 ml. The most prevalent bacteria observed in this study were *Bacillus* spp., *Pseudomonas* spp., *Arthrobacter* spp., *Enterobacter* spp., *Staphylococcus aureus* and *Escherichia coli*.The genera isolated are common and many of the individual species are able to grow on petroleum hydrocarbon. The fungal genera identified were *Aspergillus* spp., *Fusarium* spp., *Penicllium* spp., *Candida* spp.and *Saccharomyces* spp.

**Conclusion:** From this study, it was observed that POME could have a positive effect if discharged properly since little application of the effluent can enhance microbial proliferation which increases soil fertility. Most of the physicochemical parameters like BOD, TDS, Oil and grease, etc were above the limits of surface water disposal and land application by FEPA,1991 except sulphate. The organisms isolated in this study that utilized the components of the effluent might not be denitrifiers but are obviously nitrate utilizers.

*Keywords: Palm oil mill effluent; omuahunwo; physicochemical parameters.*

## **1. INTRODUCTION**

Oil Palm production is rising swiftly and has become a very signficant agriculture-based industry in several countries like Malaysia and Indonesia.Nevertheless, wet process of palm oil milling consumes a huge quantity of process water. It is estimated that about 5-7.5 tonnes of water is required for the production of 1 tonne of crude palm oil but more than 50% of the water will end up as palm oil mill effluent (POME) [1]. Oil palm is the oil that is contained in oil palm fruit pulp. The fruit attached to a rachis or an empty bunch, the assembly forming the big cluster or bunch. These bunches are the raw materials used in palm oil mill. They are harvested from oil palms in equatorial regions. Bunches are raw materials of agriculture origin with variable characteristics. The fruit is fragile and perishable so its quality depends to a great extent on the conditions of growth, harvest and transport to the factory, which are the responsibilities of the mill operator [2].

Palm Oil Mill Effluents (POME) in Nigeria are of various mostly from palm oil processing and refining mills. Palm oil is extracted from palm oil fruit mesocarp [3]. The two principal effluent (liquid wastes from palm oil mills are the clarification sludge and the sterilizer condensate, which are discharged as oily, brown/liquids at temperatures between 75°C and 85°C [4]. Palm oil mill effluents are high volume liquid wastes that have

unpleasant odour. They are predominantly organic in nature and are highly polluting. In practice, these effluents contain both dissolved and fine suspended matter, some being colloidal, and others residual oil. Its Biochemical Oxygen Demand (BOD) is fairly high, slightly acidic in reaction and consists of around 95 – 99% water, 1–4% material variously in solution and suspension and  $0.5 - 1\%$  residual oil [5]. These effluents are difficult to treat because the organic matter is essentially made up of lipids and cellulosic materials, which are not readily biodegradable.In the past, the effluent had been simply discharged into the sea or river without treatment [6]. Effluents affect the soil properties, thereby inhibiting crop growth. The higher amount of salts and high concentration of sodium and alkalinity in the wastewater generated from the industries increase the exchangeable sodium percent (ESP) of the soil to a harmful level during land disposal. The effect of high ESP is manifested by soil permeability [7].

Soil productivity is decreased as the population microorganisms (bacteria, Actinomycetes and free-living N-fixing bacteria) decrease due to irrigation by well waters polluted with discharge of industrial effluents [8]. Once the microbial population is affected, the soil productivity decreases resulting in reduced nutrient availability, plant production and the soil becomes sterile. Nitrogen nutrition of plant is affected due to the inhibition of Nitrogenfixing organisms activities and nitrification rate [8].

The biological oxidation of ammonium to nitrite in soil is primarily facilitated by two groups of chemolithotropic bacteria: Ammonium oxidizers and nitrite oxidizers. Significant alteration of the dynamics and balance of the soil nitrogen pool may occur as a result of the inhibition of either of these two groups. These nitrifying bacteria are of critical importance in nitrogen cycle in nature. The form and concentration of nitrogenous compounds in the soil sometimes controls the productivity of soil. Nitrification is carried out mainly by microbiological agents, the most important of which are the chemolithotropic nitrifying bacteria typified by the ammonia oxidizing genus, *Nitrosomonas* and the nitrite oxidizing genus, *Nitrobacter.* These organisms are considered to be the most important nitrifiers but their appearance may simply represent successful competition with other nitrifiers such as *Aeromonas hydrophilia,* which can reduce nitrite aerobically by assimilatory reduction via nitrite to ammonia [9]. The aim of this study was<br>to assess the Physicochemical and to assess the Physicochemical and microbiological quality of POME and soil in Aluu, Rivers State.

## **2. MATERIALS AND METHODS**

Palm oil mll effluent (POME) samples used in the study were obtained from two local palm oil  $\frac{d}{60}$ ml factories located at Omuahunwo in Aluu near Choba, University of Port Harcourt in Rivers State. The effluent samples from the factories were collected from the drum containing the wastewater with sterile wide mouthed screw capped bottles. These local factories use crude and manual means of production so the containers were filled to about two-third of their volume via the use of sterile smaller containers. The samples were taken to the laboratory for various analyses. Replicate samples were collected bimonthly from the same source. If there is the impossibility of immediate analysis,  $T<sub>he</sub>$ they were refrigerated.

Soil samples were obtained from an uncultivated farmland about 2 km away from the factory and a control soil sample devoid of POME contamination was taken from Department of Applied and Environmental Biology Farmland, Rivers State University, Port Harcourt in Rivers State.

Samples of 0- 15 cm depth from an uncultivated farmland and control soil (5 kg each) were collected by excavation using a sterile spade.The collected sample was put into a polythene bag. Samples were obtained from polluted soil (with POME) in proximity to the two factories. The soil samples were taken to the laboratory and stored room temperature, if not analyzed immediately.

#### **2.1 Physicochemical Analysis of Effluent and Soil Samples**

The physicochemical parameters were measured using standard analytical procedures [10]. The pH was measured using Hach pH meter (Model EC1O) and electrical conductivity was measured using Hach conductivity meter (Model CO150). Nitrate content was determined using the macro Kjeldahl digestion method of [11] and available phosphorus was determined using the method reported by [12]. Sulphate was determined using the turbidometric method. Standard methods were used for the determination of Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), ammonia, oil and grease, exchangeable cations, total dissolved and suspended solids [10].

#### **2.2 Determination of Oil and Grease**

The method was adopted from [13]. The soil samples were air dried and sieved. Ten grams of the air dried sieved samples were weighed into glass bottles and 20 ml of tetrachloroethylene was poured into the glass bottles. These bottles were placed into a shaker and maintained at room temperature. The system was allowed into a 20ml glass bottle using a glass funnel clogged with cotton wool on which anhydrous sodium sulphate was placed. Analysis of the samples was done using Hach DR4000 spectrophotometer.

#### **2.3 Determination of Exchangeable Cations**

method for the determination of exchangeable cations was adopted from [14]. The soil samples were first extracted using IN ammonium acetate solution. This was done by weighing 5 g of sieved air dried samples and adding to 30 ml of the extracting solution in a tube. This was shaken on a mechanical shaker for two hours. They were then centrifuged for five minutes and the supernatant carefully decanted into a 100 ml volumetric flask. This was then made up to the mark with the extracting solution. The exchangeable cations (Na, K, Ca<sup>2+\*</sup>, Mg<sup>2+</sup>) of the extract were determined using Unicam Atomic Absorption Spectrophotometer, Model 969.

#### **2.4 Microbiological Analyses of Effluent and Soil Samples**

#### **2.4.1 Enumeration and Identification of bacteria and fungi**

Effluent and soil samples were used for the enumeration of total Heterotrophic Bacteria (THB). Samples were serially diluted and an aliquot from each sample was placed on nutrient agar medium (Oxoid) for isolation of THB with the addition of 50 μg/ml nystatin to suppress the growth of fungi. Plates were incubated at 30°C for 24 hours before the colonies were counted. The bacterial isolates were characterized using microscopic techniques (Gram staining) and biochemical tests [15,16].

Acidified potato dextrose agar plates containing streptomycin (1 mg/100 ml) were used to obtain fungal isolates. The plates were incubated at 30°C and observed after 48 hours for yeasts and 96 hours for mould, after this, isolation of pure isolates was done [15,16].

#### **2.4.2 Estimation of Coliform and Faecal Coliform Bacteria in the POME samples**

Coliform bacteria in the POME samples were estimated using the Most Probable Number (MPN) technique. Reactions to MPN technique and thermo tolerant coliform bacteria MPN index/100 ml of each water sample was done using double strength Mac Conkey broth for 10 ml of sample and single strength Mac Conkey broth for 1ml and 0.1 ml of the sample . The test for the estimation of coliforms involves the following steps: Presumptive, confirmatory and completed test. It was performed as described by [17,16].

#### **2.4.3 Enumeration and Identification of Ammonia-oxidising bacteria (***Nitrosomonas***)**

Three milliliter of ammonium calcium carbonate medium was dispensed into tubes and sterilized Mine<br>by autoclaving at 121°C for 15 minutes. About with by autoclaving at 121°C for 15 minutes. About 0.1 ml, 0.01 ml and 0.001 ml of the effluent samples were incubated in triplicate tubes. Uninoculated control tubes were left and incubation was done at room temperature  $(28\pm2\degree C)$  for 4-9 days.

Measured weight of the soil sample (about 5 g) was dispensed in 10 ml sterile distilled water. About 0.1 ml, 0.01 ml, 0.001 ml of the soil solution was inoculated into sets of tubes of ammonium calcium carbonate medium. ammonium calcium carbonate medium. Incubation was done at 28±2°C for 14-21 days. After incubation, test for ammonia  $(NH<sub>3</sub>)$  was done by adding 3 drops of zinc-iodine-starch solution in each test tube. Positive test showing presence of  $NH<sub>3</sub>$  oxidizers were indicated by an immediate change in color to blue. The number of tubes showing blue was noted. Reference was made to MPN table (Mc Crady table) for the total number of NH<sub>3</sub>-oxidisers (Nitrosomonas) and was converted to the number per 100 ml by multiplying with the dilution factor. The result for the soil sample was reported as number per gram of soil [4].

#### **2.4.4 Enumeration and identification of Nitrite-oxidising bacteria (***Nitrobacter***)**

Five milliliter of nitrite-calcium carbonate medium was dispensed into tubes and sterilized by autoclaving at 121°C for 15 minutes. About 5 g of the soil sample was dissolved in 10 ml of sterile deionized water and 10- fold serial dilution was done to  $10^{-3}$  and 1ml was also used to inoculate free  $NO<sub>2</sub>$ -calcium carbonate medium. The tubes were incubated at room temperature (28±2°C) for 14-21 days and results were determined and reported as number per gram of soil.

Another set of inoculation was made into set of tubes of  $NO<sub>2</sub>$ -calcium carbonate medium using the effluent sample. Presence of  $NO<sub>2</sub>$  in each tube was tested with 5 drops of Griess illosvay's reagent I and II. Absence of purplish colour indicated positive result for *Nitrobacter.* Further confirmation was done with diphenylamine. Cherry-red colour indicated presence of *Nitrobacter.* Reference was made to an MPN table (McCrady table). The total number of *Nitrobacter* in each tube and probable number per 100 ml of sample was obtained by multiplying the number with the dilution factor [4].

#### **2.4.5 Enumeration and identification of Sulphate (SO4)-reducers**

Mineral agar medium agar selectively enriched sodium thioglycollate and ferrous ammonium sulphate was used. Inoculation of soil samples was by the spread plate method. All the plates were inoculated within  $1 - 4$  hours after the agar had solidifed to prevent saturation with oxygen.

To prevent moisture condensation on Petridish covers, 9cm Whattman sterile filter papers were placed on the covers until about  $10 - 15$  minutes after agar solidified. The plates were incubated and inverted in a gas jar at room temperature for 2 – 7 days. Growth and blackening around the colonies showed typical sulphate-reducing bacteria [4].

#### **2.4.6 Enumeration and identification of lipolytic bacteria**

Total lipolytic bacteria of soil samples were enumerated using an aliquot of the appropriate dilutions of the samples on pre-dried egg yolk agar medium. Incubation was done at room temperature  $(28 \pm 2^{\circ} \text{C})$  for 48 hours. Colonies surrounded by clear zones were recorded [4].

#### **2.4.7 Enumeration and identification of proteolytic bacteria**

Total proteolytic bacteria of soil sample were enumerated using an aliquot of the appropriate dilutions of the samples on milk medium. Incubation was done at room temperature (28±2°C) for 48 hours. Colonies surrounded by clear zones were recorded [4].

#### **2.4.8 Enumeration and identification of amylolytic bacteria**

Enumeration of amylolytic bacteria from the soil samples was done by inoculating pre-dried starch agar medium. Incubation of the inoculated prese plate was done at room temperature for 72 hours. At the end of the incubation period, the plates were flooded with iodine solution. Colonies with clear zones were recorded, i.e. absence of blue-black colour around the zones surrounding the colonies (clear zones).This is an indication of starch hydrolysis.

The inocula for the various tests were prepared by inoculating sterile nutrient broth contained in test tubes with isolates picked from the slants. The test tubes were incubated at  $37^{\circ}$ C for 18 – 24 hours.

For each test, controls, which consisted of inoculated media, were set up and treated the same way as the samples. Each test sample was in duplicate [4].

## **2.5 Maintenance of Pure Isolates**

Bacterial colonies were repeatedly transferred to freshly prepared nutrient agar plates by the streak-plate method and allowed to grow for 48 hours before stocking. Similarly, distinct fungal colonies were sub-cultured repeatedly on freshly prepared Sabouraud Dextrose Agar plates. Other isolates followed the same procedure. Pure isolates of the microorganisms were maintained on agar slants as stock, which were preserved in the refrigerator for further use.

#### **2.6 Characterization and Identification of Isolates**

Several methods were used to characterize and identify the isolates [15,18,19]. The test results for bacteria were evaluated using Bergey's Manual of Determinative Bacteriology [18]. Representative colonies of fungal isolates were characterized and identified based on their cultural and morphological features. The characterizations were achieved through staining techniques using lactophenol in cotton blue [18]; [19].

## **2.7 Statistical Analysis**

Results were analyzed using analysis of variance (ANOVA) at  $p \le 0.05$  and means were seperated using Duncan's multiple range Test.

## **3. RESULTS OF PHYSICOCHEMICAL CHARACTERISTICS**

The characteristics of the effluent samples are presented on Table 1. The results showed that<br>most of the effluent physicochemical of the effluent physicochemical characteristics did not fall within the limit of the Federal Environment Protection Agency [20] guidelines for wastewater discharge into surface water and for land application.

Palm Oil Mills A and B had phosphate concentration levels below FEPA limit for surface water disposal and land application (2.53 mg/l and 2.58 mg/l as against 5 mg/l). Oil and grease had concentration levels of 165853 mg/l and 165900 mg/l; sulphate, 28.87mg/l and 28.90 mg/l; nitrate, 68.83 mg/l and 68.90mg/l, ammonia, 0 mg/l for both Palm Oil mills; total suspended solids, 120200 mg/l and 122000 mg/l; total dissolved solids, 5200 mg/l and 5226 mg/l. All the parameters were beyond [20] limit for surface water disposal and land application except sulphate.

The Physicochemical Characteristics of Soil Samples from Palm Oil Mills A and B are shown on Table 2.



**Table 1. Physicochemical characteristics of effluent samples from palm oil mills A and B**

#### **Table 2. Physicochemical characteristics of soil samples from palm oil mills A and B**



#### **3.1 Microbial Analyses**

Table 3 shows the mean population of microorganisms in the effluent. The mean total heterotrophic bacterial (THB) population was 8.25 x 10<sup>5</sup>Cfu/ml for palm oil mill A and 6.1 x 10<sup>5</sup>Cfu/ml for palm oil mill B. The filamentous variat fungi (3.6 x 10<sup>3</sup> Cfu/ml and 2.5x 10<sup>3</sup> Cfu/ml) total f yeast (3.2 x 10<sup>2</sup> Cfu/ml and 2.0 x 10<sup>2</sup> Cfu/ml) and total coliform population were 12MPN/100ml and 10 MPN/100ml) respectively for palm oil mills A and B.

Total 673.6763 15

Fig. 1 shows the microbial population in the soil sample 2 km away from POME factories, control soil devoid of POME and soil polluted with POME sites A and B. The soil samples had  $8.5 \times 10^6$ Cfu/g and  $8.0 \times 10^6$  Cfu/g of total heterotrophic bacteria for factories A and B. There was a great variation of the soil sample 2km fom both factories in comparison to the control though a slight difference was observed between the polluted soil and soil sample 2km fom both factories which could be as a result of the deposition of the crude effluent in the soil.









**Fig. 1. Microbal population in soil samples in various locations**

Fig. 2a. shows the population of *Nitrosomonas* spp. in various locations. Site A had the highest population of *Nitrosomonas* spp.

Fig. 2b shows the population of Nitrobacter sp. in various locations with site A having the highest population.



**Fig. 2a. Population of** *Nitrosomonas* **spp. in various locations**



**Fig. 2b. Population of** *Nitrobacter* **spp. in various locations**

A total of twelve(12) genera of bacteria and fungi were enumerated, which include *Bacillus* sp., *Pseudomonas* sp., *Arthrobacter* sp., *Enterobacter* sp., *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus* sp., *Fusarium* sp., *Penicllium* sp., *Candida* sp. and *Saccharomyces* sp.

## **4. DISCUSSION**

The result of the physicochemical characteristics and the microbial population of the effluent showed that the effluent from both Palm oil Mills had high microbial counts. The high microbial count of the effluent samples is shown by the high BOD, total dissolved solids (TDS), nitrate and oil and grease values. High BOD values reflect large amounts of degradable organic gastrointe<br>materials in a sample of wastewater [21]. Direct observed materials in a sample of wastewater [21]. Direct release of crude industrial wastewater may have great influence on the physicochemical and biological characteristics of the soil. The level of pollutant from POME differs with the quality of the raw material and production process used to produce the palm oil.

It was observed that there was an increase in the fungal, total heterotrophic, lipolytic, proteolytic and amylolytic bacterial counts as well as other microbes from the soil polluted with palm oil mill effluent in comparison with the soil obtained 2km from polluted sites. This increase was due to the high hydrocarbon content of the palm oil mill effluent. The control soil had the lowest microbial counts since it is devoid of POME which enriched the polluted soil over time [22]. The most

prevalent bacteria species observed in this study were *Bacillus* spp., *Pseudomonas* spp., *Arthrobacter* spp., *Enterobacter* spp., *Staphylococcus aureus* and *Escherichia coli*.The genera isolated are common and many of the individual species have the ability to grow on petroleum hydrocarbons [23]. The fungal genera identified were *Aspergillus* spp., *Fusarium* spp., *Penicllium* spp., *Candida* spp. and *Saccharomyces* spp. These were in line with works of [24]. The international standards for drinking water states that potable water should not contain 100 cells of Total Heterotrophic Bacteria per 100 ml of water but unfortunately, the bacterial counts obtained in the POME from both factories exceeded the standard [25] which could pose threat to public health causing gastrointestinal diseases. In this study, it was that the physicochemical characteristics of POME-polluted soil underwent alteration after POME was discharged. Hence, POME could be ecofriendly and non-hazardous since its discharge on the soil does not cause any damage, but appreciably and significantly increases the soil nutrient levels needed for plant growth [26]. POME increases organic carbon, total nitrogen, phosphorus, potassium and magnesium levels as well as the pH level of the soil to the range of maximum nutrient availability [27]. Studies have shown that when raw POME is released into the soil environment, the pH is acidic but gradually increases to alkaline as biodegradation takes place [28]. Increase in available phosphorus content is as a result of the high absorption in the soil or a possible precipitation of phosphate in the soil, as well as the gradual biodegradation of POME, which leads to a delay effect on the soil [29]. Ths increase in POME-polluted soil could be attributed to the rise in pH level to the range of nutrient availability. Increase in Ca, Mg, K and Na content in POME-polluted soil in comparison with the control showed improvement of the soil quality. Several Researchers observed similar results and attributed the increase to the addition of POME to the soil, hence, increase in exchangeable bases levels [30,29]. POME can be used as fertilizer or animal feed substitute in terms of providing sufficient mineral requirements because of its fertilizing property. It can also be used as supplements (organic manure) to improve soil fertility. POME has been shown to be acidic in nature and it is advisable that it undergoes some form of treatment before use as manure or on land taking into consideration the physicochemical properties of the land in that exact environment. It can be used by farmers when properly treated and packaged in rural and urban areas to improve soil fertility thereby increasing the agricultural productivity for global, national and regional food demands. The treatment aids in preventing the preliminary harsh effect of POME on soil meant for agriculture. The only conspicuous problem of POME is its clogging and water logging nature which leads to death of vegetation. The application of POME by sprinkles suppresses or kills soft weeds on the ground within few days and takes about 2-3 months to regenerate [29].  $\overline{1}$ . This can be prevailed over by controlling the release or application of minute quantities of POME at a time. The condition of the soil in that environment will establish the paramount treatment for the effluent to be discharged on it [31]. Analysis of variance (ANOVA) on the data obtained showed that there was no significant difference at p ≤0.05 between the microbiological<br>(bacteria veast mould lipolytic protecutic 2. (bacteria, yeast, mould, lipolytic, proteolytic, amylolytic and sulphate reducing amylolytic and bacteria) characteristics in the POME and soil samples. There was also no significant difference at p ≤0.05 between the control, 2km from the factories and sites A and B. Using ANOVA, there was no significant  $4$ . difference in the physicochemical characteristics in the POME samples from the two factories.

## **5. CONCLUSION**

From this work, it was observed that POME  $6.$ could have a positive effect if discharged properly since little application of the effluent can

enhance microbial proliferation which increases soil fertility. Therefore, the government should create awareness to people involved in palm oil processing (both small and large scale) on the reqirement for proper disposal of effluent because if not properly managed, it can negatively affect soil fertility by hindering microbial proliferation.The organims isolated in this study that utilized the components of the effluent might not be denitrifiers but are obviously nitrate utilizers. Oil and grease was found to be a major polluting component of this effluent. The effluent encouraged the growth of lipolytic bacteria; hence, they could be employed to degrade the oil and grease to an appreciable level before final discharge into the soil environment.

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#### **COMPETING INTERESTS**

Author has declared that no competing interests exist.

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