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Physicochemical Characterization of Palm Mill Oil Effluent and Bioremediation of Impacted Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RUBE, UOE, UME and OOE designed the study. Authors UOE and OOE performed the statistical analysis. All authors wrote the protocol, and wrote the first draft of the manuscript. Authors OOE, UOE and RUBE managed the analyses of the study. Authors OOE, RUBE and UOE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Palm oil mill effluent (POME) is a waste product from palm oil production which is known to have adverse environmental effect when disposed untreated. The aim of this study was investigate the bioremediation potentials of indigenous bacteria and fungi of POME on impacted soil. Collection of samples, physicochemical characterization of POME and soil samples, microbiological analysis of the POME and lipase assay were all carried out using standard techniques. Replicate readings were then subjected to analysis of variance (ANOVA). A total of 7x4 (28) experiments designated A to G were set up. Three experiments from each block received 100 ml, 200 ml and 300 ml of POME while the fourth had no POME. The treatments were as follows: block A (soil only), B (soil +effluent), C(soil +effluent + fungi), D(soil +effluent+ bacteria), E(soil +effluent + bacteria and fungi), F(soil +effluent + chicken droppings) and G **(**soil +effluent + bacteria, fungi and chicken droppings). After

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14 days, water leaf (*Talinum triangulare)* were planted in all the set ups. Results of the physicochemical analysis of the POME and soil samples showed that addition of POME brought about significant ($p < 0.05$) changes in these parameters. A total of seven isolates were recovered from the effluent out of which *Bacillus* species and *Aspergillus niger* showed the highest lipase activity and were used for the bioremediation. Despite receiving about 300 ml of raw effluent, set ups (F and G), showed the best growth even much better than the A that had no effluent in them. Analysis of variance of the replicate readings of the plant heights showed significance ($p < 0.05$). However, the lengths and widths of the leaves of F and G performed better than the rest. The findings suggest that palm oil mill effluent impacted soil could be reclaimed using a consortium of indigenous microorganisms and biostimulation.

Keywords: Bioremediation; Talinum triangulare; Bacillus; Aspergillus niger; POME; physicochemical.

1. INTRODUCTION

The Nigeria economy is still crude oil based [1] unlike the Malaysian economy that has enjoyed a phenomenonal palm oil boom that has culminated in about 41% of the world export of palm oil. Palm oil (*Elaeis guineensis*) is a very versatile crop and nutritionally, it has been shown to contain vitamins A and E, cholesterol free and trans-free fats, saturated and unsaturated fats. In addition, it has a better shelf life than most vegetable oils. Furthermore, it is used as the basic ingredient in cooking and raw material in the cosmetic industry [2-4]. In Nigeria and particularly in Southern and Eastern Nigeria, palm oil production is largely carried out by cooperative societies and individual where it plays an important role in their economies [5] especially now that a litre of palm oil is even more expensive than a litre of crude oil. Given these nutritional and economic benefits, palm oil production has been on the increase and this has lead to high levels generated waste water popularly called palm mill oil effluents (POME).

POME remain the largest and most significant pollutant from oil mills [5,6]. Palm oil production is very water intensive. One tonne of palm oil requires five to seven times more tonnes of water of which about 50% will ends up as POME [7]. It is the copious, viscous, brownish liquid waste that emanates during the production of palm oil. Studies have shown that it contains about 90-96% water, soil particles, 0.6-0.7% residual oils and 4-5% total and suspended solids. In addition, it is acidic (pH 4-5), has a high temperature, nontoxic and high in organic contents (COD 50,000 mg/L and BOD 25,000 mg/L and 4,000-6,000 mg/L of oil and grease), and come with a very unpleasant odour [8-11].

Palm oil mill effluents have been indiscriminately disposed in producing parts of Nigeria for decades now, but it is only recently that their deleterious effects on the environment has received attention [12]. It has been shown to have the ability on over application on soil, to create anaerobic condition via the formation of an impervious coat of organic matter on the soil surface [13]. In a more recent study, POME was shown to impact negatively microbial groups such as total heterotrophic, phosphate-solubilizing, nitrifying, lipolytic, cellulolytic, and palm oil degrading bacteria. Furthermore, they showed that it could also impact levels of enzymes such as acid phosphatase and lipase activities [14]. Elsewhere, it has been shown that it can also impact the levels of nitrate, zinc, BOD and COD of nearby soils and rivers in south western Nigeria and even air quality [15, 16]. Around the oil mills in Etim Ekpo, untreated POME disposal has lead to loss of vegetation and biodiversity.

In order to cope with stringent disposal methods, it is therefore not surprising that a number of POME treatments methods have been developed in Malaysia. Some of them include anaerobic/facultative ponds, tank digestion and mechanical aeration, tank digestion and facultative ponds, decanter and facultative ponds and physic-chemical and biological treatments [7]. Interestingly, bioremediation is adjudged to be the best method for the removal of POME compared to the aforementioned treatment methods because bacteria, fungi and molds can elaborate useful extracellular enzymes needed to utilize POME [17]. In a recent study, it was shown that *Bacillus subtilis, Pseudomonas aeruginosa* and *Aspergillus niger* were all capable of biodegrading POME with *P. aeruginosa* being the most efficient [17]. In another study, it has been shown that *Candida rugosa* and *Geotrichum candidium* have great potentials in the treatment of POME impacted soils. Okwute and Ijah [18] using indigenous microorganisms and organic wastes such as cow dung and chicken droppings achieved bioremediation of POME impacted soils.

In Malaysia, stringent regulations by regulating agencies have resulted in the treatment of generated POME before their disposal into the environment [19]. However, in Nigeria, there are no such strict regulations on POME disposal. As a result, POME generated from oil mills are usually disposed directly into the environment mainly soil and also nearby aquatic habitats without any prior treatments, despite the established environmental concerns. Given the ability of microorganisms to degrade POME, the aim of this research was to characterize POME from Etim Ekpo palm oil mill physico-chemically and microbiologically, bioremediate impacted soil, and ascertain the restoration of the treated soil samples by planting water leaf (*Tallinum triangulare)*.

2. MATERIALS AND METHODS

2.1 Location of Study

This study was carried out at the Microbiology Department, Obong University located in Etim Ekpo Local Government Area of Akwa Ibom State, Nigeria.

2.2 Palm Oil Mill Effluent (POME) Collection and Preservation

Raw fresh palm oil mill effluent (POME) samples were collected from a local palm oil mill factory in Obong Ntak community using a sterile glass container and transported to the laboratory using an ice box. Some portion of the sample was immediately analysed microbiologically and physico-chemically while the rest were preserved at 4ºC to prevent microbial degradation of the POME waste water.

2.3 Soil Samples Collection

The pristine soil sample used in this study was collected from the University campus while impacted soils were obtained from the study oil mill. These were done as previously described [20].

2.4 Chicken Droppings Collection

The chicken droppings were collected fresh from a poultry house in Obong Ntak community in polythene bags and transported to the laboratory immediately. The chicken droppings were sun dried for 48 hours.

2.5 Source of Water Leaf and Identification

Water leaf used in this study was collected from the school community.

2.6 Physicochemical Characterization of the Samples

This was done as described by American Public Health Association (APHA) [21]. The samples analysed for physicochemical parameters were the pristine soil sample, soil with different volumes of effluents and the effluent. The parameters examined include pH, organic carbon, total nitrogen, available phosphorus, calcium, magnesium, sodium, aluminium, hydrogen ion, cation exchange capacity (CEC), base saturation (BS), clay, silt, sand for the impacted and pristine soil samples. For the effluent, the parameters examined were turbidity, biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), electrical conductivity (EC), nitrate, phosphate and total solids.

2.7 Biochemical Characterisation and Identification of Microbial Isolates

The isolates were identified and characterized using methods already described [22, 25]. The probable fungal isolates were *Aspergillus niger* and *Penicillium species* while the bacterial isolates were *Bacillus species, Pseudomonas species, Corynebacterium, Micrococcus* and *Staphylococcus aureus*. Briefly, the soil sample from the oil mill was serially diluted and plated on nutrient agar plates which were incubated at 37ºC for 24 hours. After incubation, the isolates were then purified, identified and inoculated onto Tributyrin agar in triplicates and incubated at 37ºC for 48 hours. The isolates were then subjected to lipase assay to select the isolates with the highest activity.

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2.8 Lipase Activity

This was done as previously described but with some modifications [26,27]. Briefly, for the lipolytic bacteria, the tributyrin agar was incorporated with Amphotericin B (to inhibit fungal growth) and for the lipolytic fungi the medium was incorporated with chloramphenicol (to inhibit bacteria growth). Holes were then bored on the solidified tributyrin agar plates. The isolates were adjusted to 0.5 Mc Farland standard before use. Zones of clearance were then measured and interpreted. The highest lipase activities were shown by *Bacillus species* and *Aspergillus* species.

2.9 Experimental Design

A total of 28 experiments arranged in blocks of 7 containing 4 experiments each were designed. Three experiments from each block received 100 ml, 200 ml and 300 ml, respectively while the fourth received no POME. Briefly, the experiments were arranged as follows. Block A**:** Soil, **B:** Soil +Effluent, **C:** Soil +Effluent + fungi, **D:** Soil +Effluent+ bacteria, **E:** Soil +Effluent + bacteria and fungi, **F:** Soil +Effluent + chicken droppings and **G:** Soil +Effluent + bacteria, fungi and chicken droppings. The bacteria and fungi used were *Bacillus species* and *Aspergillus niger*.

2.10 Performance of the Water Leaf

Water leaves were planted on all the set up exactly 14 days after the POME was spilled into the soil samples. The performance of the water leaf in experiment was followed daily for a period of four weeks after planting. At the end of four weeks, the length and width were measured.

2.11 Statistical Analysis

Replicates reading for physicochemical parameters and the heights of the water leaf were subjected to analysis of variance (ANOVA) at 95% level of significance. Probability values less than 0.05 were considered significant.

3. RESULTS

The results of the study are presented in the Figs. (1 to 5) and Tables 1 to 4. Fig. 1 shows a portion of the POME collected from the palm oil mill. Figs. 2 to 4 show the growth performance of water leaf at week 4, 6 and 8, respectively. Fig. 5 shows the widths and lengths of the leaves at week 4. In Fig. 2, the set up were arranged as thus G, F, A, E, D, C and B. On other hand, Figs. 3 and 4 were captured from B to G. The results presented in Figs. 2, 3 and 4 shows that the set up G (Soil +Effluent + bacteria, fungi and chicken droppings), F (chicken dung + effluent) and A (soil: control) had the best performance compared to the rest of the set-ups. At week six, the heights of each stem was almost double that which was obtained at week 4. At week 8, the leaves started turning yellow on all the set-ups while they became really stunted on F. A total of seven (7) microorganisms were isolated and identified as *Pseudomonas sp, Micrococcus spp, Bacillus spp, Corynebacterium spp* and *Staphylococcus aureus, Aspergillus niger and Penicillium spp.* These isolates were subjected to lipase assay and isolates (*Bacillus* and *Aspergillus niger*) with the highest activity selected for the bioremediation.

Table 1. Enumeration of lipase producing isolates

Organisms	Sizes	Results
Corynebacterium species	4.5cm	++
Pseudomonas species	3.8cm	++
Bacillus species	7.8 cm	+++
Staphylococcus species	3.6cm	++
Micrococcus	2.4cm	+
Aspergillus niger	5.2cm	+++
Penicillum	4.0cm	++

Key: 1-2.9 +; 3.0-4.9 ++; 5.0-6.9 +++; 7.0 Above ++++.

Table 2 shows the physicochemical characterization of the POME while Table 3 shows that of the pristine soil samples and the different volumes of effluent that were used to impact the soils. From the results, it can be seen that the POME under study had very low BOD 0.13±0.00 mg/L, followed by COD 0.32±0.01 mg/L and DO 0.59±0.01 mg/L. The pH of the POME showed that it is acidic with a value of 4.45. Nitrate levels were more than twice that of phosphate. Electrical conductivity (EC) was 594±1.41 µs/cm and turbidity gave a value of 123,800±141.42 NTU. Total dissolved solids were 36.00±1.41. Statistical analysis of the replicate readings showed significance ($p < 0.05$). The pH of the pristine soil samples was higher than those that received 100 ml and 300 ml of effluents. Organic carbon content of the soil was in between those of the effluent impacted samples. The nitrogen content of the samples was least in the unimpacted soil samples while the sample with the highest impacted volume had the highest. Furthermore similar results were seen for available phosphorus contents which gave the highest value of all the elemental composition analysed. Calcium, magnesium, potassium and sodium levels were almost similar for all the samples examined. However, aluminium and hydrogen ion (mol/kg) and electrical conductivity were higher in the unimpacted than the impacted samples. Base saturation was higher in the impacted samples. Physical properties of the samples on analysis showed that all the soil samples had more sand than clay and silt indicating a sandy-loam soil for impacted and unimpacted samples. Again, analysis gave a significant p value (< 0.05).

Following treatments, the growth performance of the *T. triangulare* was measured at week four and the average determined for each block (A to G) as presented in Table 4. The highest heights were attained by the treatments that received no POME in B-G. Pristine soil sample performed better than soils that received treatments except for F and G where the performance was maximum. The best performance was seen in block G, however, as the volume of effluent increased, there was a corresponding decrease in the height of the plant.

Table 2. Physicochemical parameters of effluent

Parameters	Effluent
BOD (mg/L)	0.13±0.00[a]
COD (mg/L)	0.32±0.01
DO (mg/L)	0.59±0.01
Ph	4.45.00
EC (µs/cm)	594.00±1.41
Turbidity (NTU)	123,800.00±141.42
Nitrates (mg/L)	169.00±1.41
Phosphate (mg/L)	69.12±0.01
Total solids (mg/L)	36.00±1.41

[a]Represents significant Mean±SD values that are significant (p < 0.05).
Key: BOD – Biochemical Oxygen Demand, COD – Chemical Oxygen Demand, DO –Dissolved Oxygen, EC – Electrical conductivity

Table 3. Physicochemical analysis of soil samples

Parameters	OS	OS+300 ml effluent	OS+100 ml effluent
pH	5.60[a]	5.20[a]	5.00[a]
Organic carbon (%)	0.74	0.69	0.88
Total nitrogen (%)	0.01	0.05	0.04
Available phosphorus (mg/kg)	32.12	42.00	34.37
Calcium (mol/kg)	2.00	1.80	2.00
Magnesium (mol/kg)	1.00	1.20	0.80
Potassium (mol/kg)	0.12	0.11	0.09
Sodium (mol/kg)	0.08	0.08	0.07
Aluminium (mol/kg)	0.36	0.26	0.24
Hydrogen ion (mol/kg)	2.04	1.16	1.20
CEC (mol/kg)	5.60	4.61	4.40
BS (%)	57.00	69.00	67.00
Clay (%)	8.00	4.00	4.00
Silt (%)	5.70	8.70	10.70
Sand (%)	86.30	87.30	85.30

[a]Represents replicate readings that are significant (p < 0.05) across the rows at 0.05 level of significance. OS = Ordinary soil. CEC= Cationic exchange capacity and BS= Base saturation.

Table 4. Mean Height of *T. triangulare* with different treatments (cm) of POME

Treatments	Control (o ml)	100 ml	200 ml	300 ml
Soil (A)	7.96±1.29	8.60±0.96	8.10±3.75	8.10±1.67[b]
Soil + Effluent (B)	8.32±2.81	7.00±3.34	7.52±1.53	7.02±0.13[a]
SE + fungi (C)	7.88±0.63	6.86±0.42	5.72±1.17	3.44±1 .64[a]
SE + bacteria (D)	7.18±0.75	7.18±0.25	6.70±0.76	4.75±1.48[c]
SE+fungi+bacteria (E)	12.96±2.82	8.48±1.26	7.20±0.57	6.60±0.65[c]
SE+chicken dung (F)	22.20±5.95	18.00±1.27	15.20±2.11	12.66±1.98[c]
SE+chickendung+fungi+bacteria (G)	25.80±6.18	19.72±1.75	16.70±1.48	14.20±2.19[c]

[a and b] Represents significant (p<0.05) measurements across the row and columns while c represents none significant measurements (p > 0.05).
Note: Key: fungi = Aspergillus niger and bacteria = Bacillus species and SE = soil + effluent.

Fig. 1. A portion of the collected POME

Fig. 2. Growth of Water leaf at 4weeks

Fig. 3. Growth of Water leaf at 6weeks

Fig. 4. Growth of Water leaf at 8 weeks

4. DISCUSSION

In Nigeria there are more studies on bioremediation of crude oil polluted soils than on the bioremediation of the soils polluted with palm oil mill effluent even as the price of palm oil is presently higher than that of petrol in the country. Studies have shown that physicochemical properties of POME and even soil vary from location to location [28,29]. The results of this study indicates that palm oil effluent is more acidic in pH, than the pristine soil sample examined and also lower than the 5.11 reported previously for unimpacted soil in Calabar [28]. Furthermore, in their study, they found sand, silt and clay in the proportions of 78.40, 4.43 and 17.30%, respectively and these were different to our findings of 86.30, 8.00 and 5.70%. However, both soils can be classified as sandy-loam soil because they contain at least 75% sand and thus can support the growth of palm trees [28].

On addition of the POME, the physicochemical properties of the receiving soils such as pH, organic carbon, nitrogen, phosphorus, metals, soil properties, BS and CEC changed. This agrees with an earlier study that showed that the soil contaminated with POME changed physicochemical parameters such as pH, oil and grease and even elements [29]. Eze et al. [29] reported a DO of 1.8, nitrate 0.55, phosphate 0.21, BOD 25.00, COD 60.00 and TDS 0.35 mg/L, and turbidity of 55.00 NTU and pH 7.20. When compared to our study POME, the DO, BOD, COD and pH, were lower while the levels of TDS, nitrate, phosphate and turbidity in our study were higher. The study POME physico chemical parameters, however, fall within the regulatory standard of the Federal Ministry of Environment [30].

Loretta et al. [17] showed that *Pseudomonas aeruginosa* can more efficiently degrade POME

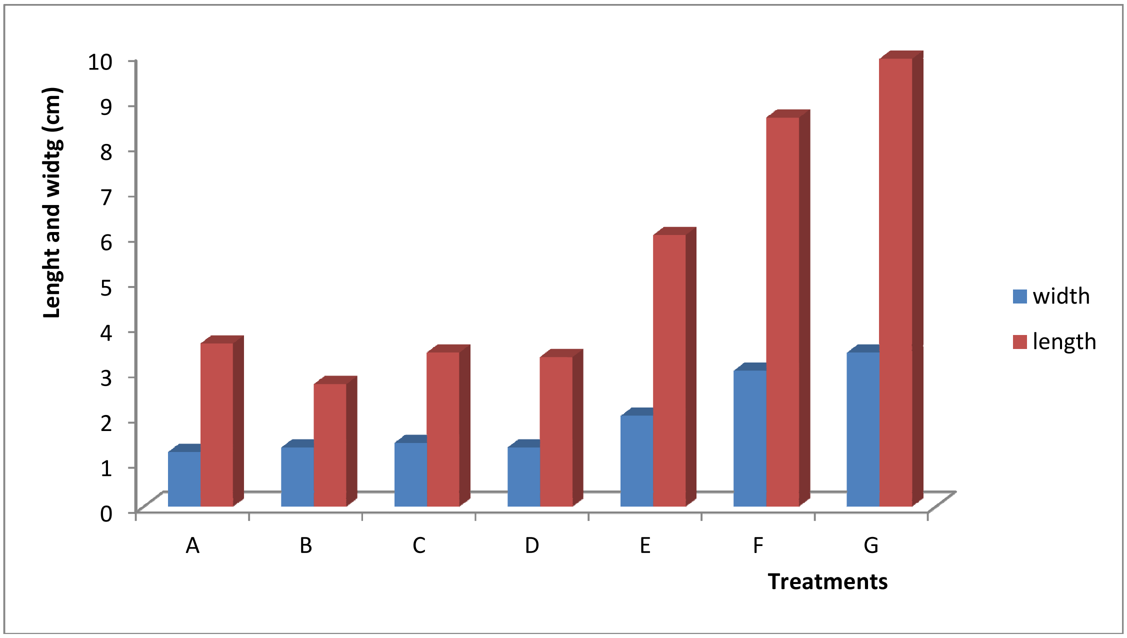

Fig. 5. Length and width of *T. triangulare* leaves across different treatments at week 4

using it as a carbon source for growth better than *Aspergillus niger* and *Bacillus species*. Interestingly, our study isolates showed a much better synergistic effect when biostimulated with chicken droppings as a source of limiting nutrients.

Eze et al. [29] isolated a total of 13 bacteria and fungi including *Bacillus* species, *Pseudomonas aeruginosa, S. aureus, Aspergillus, Penicillium* species, and *Micrococcus*, which were also isolated in our study with *Bacillus and Aspergillus* being amongst the most frequent in occurrence. Ibegbulam-Njoku and Achi [31] showed that indigenous fungi *Geotrichum candidium* and *Candida rogusa* have the ability to reduce pollution effects of POME. In another study, it was shown that indigenous microbial isolates from POME have the potential to degrade organic components and also mixed cultures were even more effective in the reduction of COD and BOD of POME [32]. This assertion of Bala et al. [32] is evident in the better growth performance shown by the experiments F and G. Despite receiving about 300 ml of raw effluent (F and G), the test vegetable grew much better than the controls that had no effluent in them. Also the presence of limiting nutrients from the chicken droppings [33] explains in part the better performance of the study plant as seen in experiments F and G. When compared to other impacted soil samples that had single isolated bacterium, E performed much better than D, C, and B especially at lower volumes of effluents.

5. CONCLUSION

The presence study has shown that POME has the ability to alter the physicochemical properties of the receiving soil samples. Furthermore, indigenous isolates present in POME have varying ability to degrade palm mill oil effluent when used singly, mixed and biostimulated with limiting nutrients from organic waste.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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