

Physicochemical characterization of palm mill oil effluent and bioremediation of impacted soil using a consortium of microorganisms and biostimulation

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 soils samples, microbiological analysis of the POME and lipase assay were all carried out using standard techniques. Replicate readings were the 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 100ml, 200ml and 300ml of POME while the fourth had no POME. Thus, 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 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 changed 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 best lipase activity and were used for the bioremediation. Despite receiving about 300ml of raw effluent, set ups (F and G), gave the best growth even much better than the A that had no effluent in them. Further analysis showed that the height of the plants was significantly ($p < 0.05$). The lengths and widths of the leaves were also better. The findings suggest that palm oil mill effluent impacted soil could be reclaimed using a consortium of indigenous *Aspergillus niger* and *Bacillus* species and biostimulation.

Keywords: Bioremediation, *Talinum triangulare*, *Bacillus*, *Aspergillus niger*, POME, physicochemical

INTRODUCTION

The Nigeria economy is still crude oil based [1] unlike the Malaysian economy that has enjoyed a phenomenon 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, basic ingredient in cooking and a raw material in the cosmetic industry [2][3]. In Nigeria and particularly in Southern and Eastern Nigeria,

40 palm oil production is largely carried out by cooperative societies and individual
41 where it plays an important role in their economies[5] especially now that a litre of
42 palm oil is even more expensive than a litre of crude oil! Given these nutritional and
43 economic benefits, palm oil production has been on the increase leading to high
44 levels of waste water generation popularly called palm mill oil effluents (POME).

45 POME remain the largest and most significant pollutant from oil mills [5][6]. Palm oil
46 production is very water intensive with about one tonne of palm oil requiring five to
47 seven times more tonnes of water of which about 50% will end up as POME [7]. It
48 is the copious, viscous, brownish liquid waste that emanates during the production of
49 the palm oil. Studies have shown that it contains about 90-96% water, soil particles,
50 0.6-0.7% residual oils, 4-5% total and suspended solids, acidic (pH 4-5), high
51 temperature nontoxic, high organic content (COD 50,00mg/L and BOD 25,000mg/L),
52 4,000-6,000 mg/L of oil and grease and a very unpleasant odour [8]-[11].

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

65 In order to cope with stringent disposal methods, it is therefore not surprising that a
66 number of POME treatments methods have been developed in Malaysia. Some of
67 them include anaerobic/facultative ponds, tank digestion and mechanical aeration,
68 tank digestion and facultative ponds, decanter and facultative ponds and physic-
69 chemical and biological treatments [7]. Interestingly, bioremediation is adjudged to
70 be the best method for the removal of POME compared to the aforementioned
71 treatment methods because bacteria, fungi and mold can elaborate useful
72 extracellular enzymes needed to utilize POME [16]. In a recent study, it was shown
73 that *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Aspergillus niger* were all
74 capable of biodegrading POME with *P. aeruginosa* being the most efficient [16]. In
75 another study, it has been shown that *Candida rugosa* and *Geotrichum candidum*
76 have great potential in the treatment of POME impacted soils. Okwute and Ijah [18]
77 using indigenous microorganisms and concentration of organic wastes such as cow
78 dung and chicken droppings achieved bioremediation.

79 In Malaysia, there exist stringent regulations by regulating agencies such that oil mill
80 owners have to treat generated POME before their disposal into the environment

[19]. However, in Nigeria, there exist no such strict regulations exists on POME disposal. As a result POME generated from oil mills are usually disposed directly into the environment mainly soil and eventually nearby water bodies without any prior treatments, despite the established environmental concerns. Given the ability of bioremediation to degrade pollutants, the aim of this research was to characterize POME from Etim Ekpo palm oil mill physico-chemically and microbiologically, bioremediate impacted soil using biostimulation and bioaugmentation, and ascertain the restoration of the treated soil samples by planting water leaf (*Tallinum triangulare*).

90

91 **MATERIALS AND METHODS**

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93 **Location of study**

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

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97 **Palm oil mill effluent (POME) collection and preservation**

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

103

104 **Soil samples collection**

105 The pristine soil sample used in this study was collected from the University campus
106 and it was done as previously described [20].

107

108 **Chicken droppings collection**

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

112

113 **Source of Water leaf and Identification**

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

116

117 **Physicochemical characterization of the samples**

118 This was done as described by APHA [21]. The samples analysed for
119 physicochemical parameters include the pristine soil sample, soil with different
120 volumes of effluents and the effluent. The parameters examined include pH, organic
121 carbon, total nitrogen, available phosphorus, calcium, magnesium, sodium,
122 aluminium, hydrogen ion, CEC, BS, clay, silt, sand for the impacted and pristine soil

samples. For the effluent, the parameters examined were turbidity, BOD, COD, DO, EC, nitrate, phosphate and total solids.

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*. The isolates were then subjected to lipase assay to screen for lipase activity.

Lipase activity

For the lipolytic bacteria, the medium were incorporated with Amphotericin B (to inhibit fungal growth) and for the lipolytic fungi the medium was incorporated with chloramphenicol (to inhibit bacteria growth). The plates were done in triplicates incubated at room temperature ($28\pm 2^{\circ}\text{C}$) for 48 hours according to methods already described [26][27]. The best lipase activities were shown by *Bacillus* and *Aspergillus* species.

Experimental design

The experiment was designed using a simple design. Briefly, a total of 28 experiments arranged in blocks of 7 containing 4 experiments each. Three experiments from each block received 100ml, 200ml and 300ml, respectively while the fourth received no POME. Thus, **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*.

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. During which, the length and width were obtained in addition to the heights of the plant in all the set ups at week four.

Statistical analysis

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

RESULTS

The results of the study are presented in the figures (1 to 5) and Tables 1 to 4. Figure 1 shows a portion of the POME collected from the palm oil mill. Figures 2 to 4 show the growth performance of water leaf at week 4, 6 and 8, respectively. Figure 5

shows the widths and lengths of the vegetable leaves at week 4. In figure 2, the set up were arranged as thus G, F, A, E, D, C and B. On other hand, figures 3 and 4 were captured from B to G. The results presented in figures 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 activity test and the best two which were *Bacillus* and *Aspergillus* were then selected for the bioremediation.

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 understudy was very low on BOD $0.13 \pm 0.00 \text{mg/L}$, followed by COD $0.32 \pm 0.01 \text{mg/L}$ and DO $0.59 \pm 0.01 \text{mg/L}$. 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 $594 \pm 1.41 \text{ }\mu\text{S/cm}$ and turbidity gave a value of $123800 \pm 141.42 \text{ NTU}$ Total dissolved solids were 36.00 ± 1.41 . Statistical analysis of the replicate readings showed significance ($p < 0.001$). Table 3 indicates that the pH of the pristine soil samples was higher than those that received 100ml and 300mls 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 non 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 (0.001).

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 height was 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 1: Enumeration of Lipase producing isolates

Organisms	Sizes	Results
Isolate 1	4.5cm	++
Isolate 2	3.8cm	++
Isolate 3	7.8 cm	+++
Isolate 4	3.6cm	++
Isolate 5	2.4cm	+
Isolate 6	5.2cm	+++
Isolate 7	4.0cm	++

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

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

^aRepresents significant Mean±SD values that are significant (p < 0.001).

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+300ml Effluent	OS+100ml 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

^aRepresents replicate readings that are significant ($p < 0.001$) across the rows at 0.05 level of significance. OS = Ordinary soil. CEC= Cationic exchange capacity and BS= Base saturation.

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

Treatments	Control (o ml)	100ml	200ml	300ml
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

222 ^{a and b} Represents significant ($p < 0.05$) measurements across the row and columns
 223 while c represents none significant measurements ($p > 0.05$). Note: Key: fungi =

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234 Aspergillus and bacteria = bacillus species and SE = soil + effluent.



Figure 1: A portion of the collected POME



Figure 2: Growth of Water leaf at 4weeks



Figure 3: Growth of Water leaf at 6weeks



Figure 4: Growth of Water leaf at 8 weeks

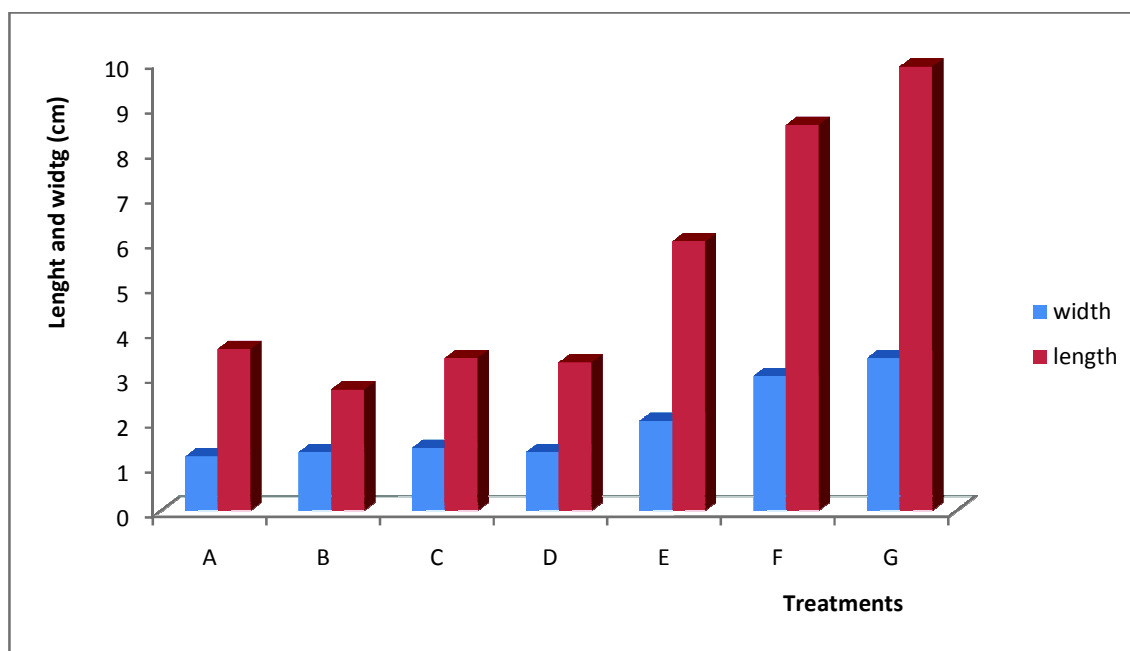


Figure 5: length and width of *T. triangulare* leaves across different treatments at week 4.

DISCUSSION

In Nigeria there exist 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 physico-chemical properties of POME and even soil varies on the 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 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 atleast 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 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, pH, were lower while the 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 as a carbon source for growth better than *Aspergillus niger* and *Bacillus species*. Interestingly, these two microbes isolated in our study have shown 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 candidum* 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 that mixed cultures are 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 300ml of raw effluent (F and G), the test vegetable grew much better than the controls A that had no effluent in them. Also the presence of limiting nutrients from the chicken droppings [33] explains in part the better vegetable performance 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.

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 organic waste.

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