

Physicochemical characterization of palm mill oil effluent and bioremediation of impacted soil

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. Analysis of variance of the plant heights showed 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, and is also the basic ingredient in cooking and a raw material in the cosmetic industry [2,3]. 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

41 that a litre of palm oil is even more expensive than a litre of crude oil. Given these
42 nutritional and economic benefits, palm oil production has been on the increase
43 leading to high levels of waste water generation popularly called palm mill oil
44 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 in organic content (COD 50,000mg/L and BOD
52 25,000mg/L), 4,000-6,000 mg/L of oil and grease and a very unpleasant odour [8-
53 11].

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

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

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

[19]. However, in Nigeria, there exist 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 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, and **ascertain the restoration of the treated soil samples by planting water leaf (*Tallinum triangulare*)**.

MATERIALS AND METHODS

Location of study

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

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. Samples were preserved at stored at 4 °C to prevent microbial degradation of the POME waste water.

Soil samples collection

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

Chicken droppings collection

The chicken droppings was 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 48hours.

Source of Water leaf and Identification

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

Physicochemical characterization of the samples

This was done as described by APHA [21]. The samples analysed for physicochemical parameters include 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, 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

This was done as previously described as previously but with some modifications [26][27]. 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 bored on the solidified Tributyrin agar plates. Briefly, the soil sample from the oil mill was serially diluted and plated on nutrient agar plates which were incubated at 37°C for 24 hours. After incubation, the isolates were then purified and inoculated onto Tributyrin agar in triplicates and incubated at 37°C for 48 hours. The isolates were adjusted to MacFarland standard before use. Zones of clearance were then measured and interpreted. 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.

166 RESULTS

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

182 Table 2 shows the physicochemical characterization of the POME while Table 3
183 shows that of the pristine soil samples and the different volumes of effluent that were
184 used to impact the soils. From the results, it can be seen that the POME understudy
185 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
186 $0.59 \pm 0.01 \text{mg/L}$. The pH of the POME showed that it is acidic with a value of 4.45.
187 Nitrate levels were more than twice that of phosphate. Electrical conductivity (EC)
188 was $594 \pm 1.41 \text{ }\mu\text{S/cm}$ and turbidity gave a value of $123800 \pm 141.42 \text{ NTU}$ Total
189 dissolved solids were 36.00 ± 1.41 . Statistical analysis of the replicate readings
190 showed significance ($p < 0.001$). Table 3 indicates that the pH of the pristine soil
191 samples was higher than those that received 100ml and 300mls of effluents.
192 Organic carbon content of the soil was in between those of the effluent impacted
193 samples. The nitrogen content of the samples was least in the unimpacted soil
194 samples while the sample with the highest impacted volume had the highest.
195 Furthermore similar results were seen for available phosphorus contents which gave
196 the highest value of all the elemental composition analysed. Calcium, magnesium,
197 potassium and sodium levels were almost similar for all the samples examined.
198 However, aluminium and hydrogen ion (mol/kg) and electrical conductivity were
199 higher in the unimpacted than the impacted samples. Base saturation was higher in
200 the impacted samples. Physical properties of the samples non analysis showed that
201 all the soil samples had more sand than clay and silt indicating a sandy-loam soil for
202 impacted and unimpacted samples. Again, analysis gave a significant p value
203 (0.001).

204 Following treatments, the growth performance of the *T. triangulare* was measured at
205 week four and the average determined for each block (A to G) as presented in Table
206 4. The highest height was attained by the treatments that received no POME in B-G.
207 Pristine soil sample performed better than soils that received treatments except for F
208 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 ++++.

Isolates 3 and 6 were identified as *Bacillus* and *Aspergillus niger*, respectively.

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.

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

^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 =

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241 *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

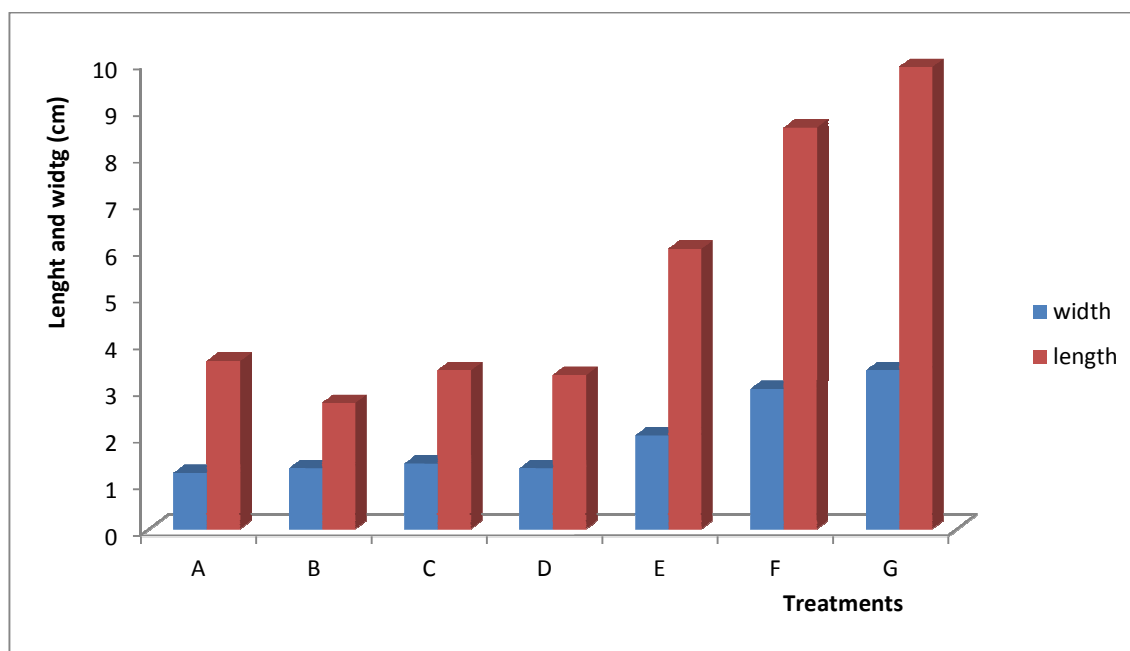


Figure 5: length and width of *T. triangulare* leaves across different treatments at week 4.

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 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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