

## The effect of Palm oil mill effluents on soil microbial community

### Consequences derived from effluents from palm oil extraction plants on the soil microbial community

Comment [FJAG1]: This is my suggestion.

#### ABSTRACT

Soils where palm oil mill effluents (POME) are being discharged were examined to evaluate the effect of the effluent on soil micro-flora and consequent soil fertility. A total of five(5) different locations, were analyzed, from where ten (10) samples were collected for analysis. Soil samples unimpacted with POME served as control. The total heterotrophic bacteria, coliform and fungal counts were evaluated using the pour plate and spreadplate method. Result showed that the Total heterotrophic bacterial, Total Coliform and Total fungal counts ( $3.8 \times 10^3$ CFU/ml,  $3.5 \times 10^3$ CFU/ml and  $3.4 \times 10^2$ CFU/ml respectively) of the impacted soils were lower compared to the values ( $3.6 \times 10^6$ CFU/ml,  $3.6 \times 10^6$ CFU/ml and  $2.6 \times 10^4$ CFU/ml respectively) of the un-impacted soils which served as control. The bacterial genera implicated in margarine production processes include; *Bacillus spp*, *Micrococcus spp*, *Klebsiella spp*, *Pseudomonas spp*, *Staphylococcus spp*, *Salmonella spp*. The fungal genera isolated from soils with palm oil mill effluents include; *Aspergillus spp*, *Rhizopus spp*, and *Mucor spp*, the results obtained indicates that palm oil mill effluents have immediate negative impact on soil microbiota as their application could lead to changes which affects soil microbial communities. Therefore, it would be advised that proper guidelines be set up for possible pre-treatment and safe discharge of palm oil mill effluents, in other to avoid its effect on soil microbiota and soil fertility.

#### ABSTRACT

In order to know the consequences of the effluent on the microflora of the soil and its respective fertility, the size of the experimental sample was defined, which was represented by five locations, from which ten portions of soil were collected for microbiological analysis. Unimpacted soil samples served as controls. Total counts of heterotrophic bacteria, coliforms, and fungi were assessed using the pour plate and spread plate method. The result showed that the counts of total heterotrophic bacteria,

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total coliforms and total fungi ( $3.8 \times 10^3$ CFU/mL,  $3.5 \times 10^3$ CFU/mL and  $3.4 \times 10^2$ CFU/mL respectively) of the impacted soils were lower compared to the values ( $3 \times 10^6$  CFU/mL,  $3.6 \times 10^6$ CFU/mL). mL and  $2.6 \times 10^4$ UFC/mL respectively) of the non-impacted soils that served as control. Bacterial genera involved in margarine production processes include *Bacillus* spp, *Micrococcus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Salmonella* spp. The genera of fungi isolated from soils with effluents from palm oil extracting plants include *Aspergillus* spp, *Rhizopus* spp and *Mucor* spp. The results obtained indicate that effluents from palm oil extracting plants have an immediate negative impact on the microbiota of the soil, since its application could cause changes that affect the microbial communities. Therefore, it is recommended that adequate guidelines be established for the possible pre-treatment and safe discharge of effluents from palm oil mills, in addition to avoiding their effect on the microbiota and soil fertility.

**Keywords:** *Palm oil mill effluent, microbial loads, soil microbiota, soil fertility.*

**Keywords:** Hyphomycetes, Mucoraceae, Bacillaceae, Micrococcaceae, Enterobacteriaceae.

Comment [FJAG3]: This is my suggestion.

## INTRODUCTION

The oil palm industry is one of the major agro-industries in some parts of the world such as Malaysia, Indonesia, Thailand, Columbia and Nigeria (Izah and Ohimain, 2016). Palm oil processing is carried out using large quantities of water in mills where oil is extracted from the palm fruits. During the extraction of crude palm oil from the fresh fruits, about 50% of the water results in palm oil mill effluent (POME). It is estimated that for 1 tonne of crude palm oil produced, 5 - 7.5 tonnes of water ends up as POME (Ahmad *et al.*, 2003). The solid waste products that result from the milling operation are empty fruit bunches, palm

fibre, and palm kernel. In both traditional and modern milling settings, these solid waste products are all put to economically useful purposes such as fuel material and mulch in agriculture.

#### INTRODUCTION

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Soil is the key component of the natural ecosystem, and as such, environmental sustainability is highly dependent on a sustainable soil ecosystem (Adenipekun, 2008; Onuh et al., 2008). Kolwan et al., (2006) defined the soil as the upper layer of the earth's lithosphere, formed from withered rock that has been transformed by living organisms. Soil is made up of five elements which are: minerals, water, soil air, organic matter and living soil organisms. Due to its chemical composition and physical properties, soil forms a habitat for massive numbers of microorganisms and other living organisms. Microorganisms found in soil include bacteria, algae, fungi, actinomycetes, protozoa, and viruses (Willey et al., 2008). The industrial and economic activities of man in his immediate environment have brought better

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living conditions for him. However, these activities produce waste that is inevitably incorporated into the soil and negatively alters the ecological balance, thus threatening human life and health (Olorunfemi et al., 2008).

Palm oil production worldwide is growing at a very high rate and pollution caused by waste materials from palm oil mills has become a serious problem (Orji et al., 2006). Effluent from palm oil mills is often dumped indiscriminately into the environment, particularly on farmland (Ogboghodo et al., 2001). POME discharged from an oil mill is objectionable and could contaminate surrounding streams, rivers, or land (Hartley, 2004). Soils where effluents from palm oil mills were recently discharged had very low microbial population and diversity (Orji et al., 2006).

The entire ecosystem changes when new materials are added to the soil, as microorganisms die or move away from contaminants. Soil microorganisms have enzymes that make them show a variety of metabolic activity, which ensures the permanence of the cycles of the elements in nature. The enumeration of microorganisms and the evaluation of the activity of soil enzymes provide an integrative measure of the biological state of the soil (Li et al., 2005). POME exists as a viscous, brownish liquid containing approximately greater than 95.0-96.0% water, along with 0.6-0.7% tar sand 2.0-4.0% suspended solids for the source of fruit remains and is known as a non-toxic substance (Mohammed and Chong, 2014). The brownish color is due to the origin of carotene (8 mg/L), pectin (3400 mg/L), tannin, phenolic (5800 mg/L) and lignin (4700 mg/L), so it is a nutrient-substance rich (Mohammed and Chong, 2014). POME is known to be a major problem in all oil palm mills due to its high abundance and disposal problems. Direct discharge of POME onto the ground results in clogging, waterlogging of the soil, and kills vegetation on contact.

The discharge of the effluent into the waterway causes water depletion, which results in aquatic pollution. In extreme cases, the river water turns brown with an unpleasant odor and a slimy characteristic. Mortality of fish and other aquatic organisms increases, and local people are deprived of local sources of water for domestic use and fishing.

A deeper understanding of the consequences generated by effluents from palm oil mills and effective techniques for their treatment are of the utmost importance, since the available information is still scarce. Therefore, this study was designed to examine the effects of POME on the soil microbiota, which is an important indicator of soil fertility.

## **MATERIALS AND METHODS**

### **Collection of Samples**

The samples used for this study, were obtained from different oil mill locations in Obubra, Cross River State, with the aid of sterile nylon. The samples were transported to Microbiology laboratory of Cross River University of Technology, Calabar for further analysis.

### **Microbiological Analysis**

#### **Enumeration of Total Heterotrophic Bacterial Counts of soil samples**

Total heterotrophic bacterial count of the soil sample with palm oil mill effluent and control was determined using pour plate method as described by Cheesbrough (2002). Serial dilution ( $10^1$ – $10^{10}$ ) were carried out from the liquid sample. Exactly one millimeter (1mL) was taken from each selected dilution ( $10^2$ ,  $10^4$  y  $10^6$ ) into

**Comment [FJAG5]:** Missing bibliographic citation

**Comment [FJAG6]:** In this section, the experimental technique used must be specified: statistical model, experimental design, number of repetitions, type and size of plot, means comparison tests, way of taking the sample, treatments evaluated, data collected, forms of evaluation, cite the taxonomy of soils studied...

**Comment [FJAG7]:** It remains to specify at what depth and the number of samples per location.

**Comment [FJAG8]:** Write properly.

sterile petri dishes. The molten sterilized Nutrient and MacConkey agar were poured into different plates, swirled to spread the inoculums evenly within the agar medium and allowed to solidify, then incubated at 37<sup>b</sup>c for 24 hours. Thereafter, plates with colony growth were counted.

Comment [FJAG9]: Write properly.

#### **Determination of Total Fungal Count of the soil samples.**

The spread plate method was used for determination of total fungi count of soil samples impacted with palm oil mill effluent and those without palm oil mill effluent (control) following the method of Cheesbrough, (2002). One millimeter (1ml) of the diluents from the samples was aseptically transferred onto sterilized, solidified Sabouraud Dextrose agar medium. Then incubated at 28-30<sup>0</sup>C for 3-4 days. Plates with colony growth were counted and expressed as colony forming unit per millimeter (CFU/g).

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#### **Biochemical Characterization and Identification of Isolates**

All bacterial isolates were characterized and identified based on their cultural, morphological, microscopic and biochemical features following the method described by Cheesbrough, (2002). Biochemical test conducted includes: Gram staining, Triple sugar iron, indole, methyl red, citrate utilization, catalase, oxidase, Voges Proskauer and sugar fermentation.

#### **Identification of fungal isolates**

Pure cultures obtained were used for standard identification. The colonial morphologies of the fungal isolates on Sabouraud Dextrose Agar were observed for colour and type of growth that is wooly or cottony. Microscopic identification was carried out by preparing wet mount using lactophenol, methylene blue to observe the microscopic characteristics of the fungi such as type of hyphae (whether

Comment [FJAG11]: Structures with taxonomic validity must be mentioned (Fialide etc...)

septate or non-septate). Both microscopic and macroscopic features of the fungal isolates were matched based on the mycological atlas for fungal identification.

## RESULTS

The enumeration of the samples analyzed, had different microbial loads. The total heterotrophic bacterial count of the samples from different locations revealed that the palm oil mill effluent has effect on the soil microbiota as the soil samples without palm oil mill effluent yielded high bacterial count when compared to soil samples impacted with palm oil mil effluent as shown in Table1.

Comment [FJAG12]: remove the comma

**Table 1:**Total heterotrophic bacterial (THB) count of samples with palm oil mill effluent and samples without effluent (Control).

Comment [FJAG13]: Insert THB after its meaning, to avoid confusion when looking at the Table.

Sample Location	THB count CFU/g (With Effluent)	THB count CFU/g (Without effluent)
Location 1	$3.3 \times 10^2$	$2.4 \times 10^7$
Location 2	$3.8 \times 10^3$	$3.6 \times 10^6$
Location 3	$3.7 \times 10^3$	$3.9 \times 10^6$
Location 4	$2.8 \times 10^3$	$3.7 \times 10^5$
Location 5	$3.1 \times 10^3$	$3.3 \times 10^6$

The total coliform count of the samples showed that the soil samples impacted with palm oil mill effluent yielded low coliform count (Table 2) when compared to the control (soil without palm oil mill effluent).

**Table 2:**Total Coliform (TC) count of Samples with palm oil mill effluent and samples without palm oil mill effluent (Control)

Sample location	TC count CFU/g (With Effluent)	TC count CFU/g (Without effluent)
Location 1	$3.1 \times 10^3$	$2.4 \times 10^6$
Location 2	$3.5 \times 10^3$	$3.9 \times 10^6$
Location 3	$2.9 \times 10^3$	$3.6 \times 10^6$
Location 4	$3.4 \times 10^3$	$3.7 \times 10^5$
Location 5	$3.0 \times 10^3$	$3.3 \times 10^6$

**Comment [FJAG14]:** Insert this abbreviation, the above helps to correctly interpret the Table.

The fugal screening also revealed that the palm oil mill effluent had a negative impact on fugal loads as the soil without palm oil mill effluent had higher fungal load compared to the soil samples impacted with palm oil mill effluent as displayed in Table 3.

**Table 3:**Total Fungal (TF) count of samples with palm oil mill effluent and samples without palm oil mill effluent (Control)

Sample location	TF count CFU/g (with Effluent)	TF count CFU/g (without effluent)
Location 1	$3.2 \times 10^2$	$2.2 \times 10^4$
Location 2	$3.4 \times 10^2$	$2.6 \times 10^4$
Location 3	$3.2 \times 10^2$	$2.1 \times 10^4$
Location 4	$2.9 \times 10^2$	$2.7 \times 10^4$
Location 5	$3.5 \times 10^2$	$3.0 \times 10^4$

Biochemical characterization and identification processes revealed as shown in (Table4), that *Salmonella* species, *Micrococcus* spp., *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli*, *Klebsiella* spp., etc. were the suspected bacterial isolates observed in the samples.

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Table 4: Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates

SN	Colour/Appearance	Gram Stain		Arrangement	TSI											Suspected Organisms		
		RXN	Shape		Catalase	Oxidase	Coagulase	Methyl red	Indole	Voges prokauer	Citrate	Glucose	Sucrose	Lactose	Slant		Butt	H <sub>2</sub> S
1.	Circular white convex	+	Cocci	Clusters	+	-	-	-	-	-	-	+	+	-	A	A	-	<i>Lactobacillus spp</i>
2.	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus spp</i>
3.	Irregular Raised milky	+	Cocci	Singles		-	-	-	-	-	+	+	+	+	A	A	+	<i>Micrococcus spp</i>
4.	Irregular milky flat	-	Rod	Pairs	+	+	-	+	-	-	+	+	+	A	A	-	<i>Escherichia coli</i>	
5.	Circular White convex	+	Cocci	Clusters	+	-	-	-	-	-	+	+	-	A	A	-	<i>Lacto bacillus spp</i>	
6.	Irregular milky flat	+	Rod	Pairs	+	+	-	+	-	-	+	+	+	A	A	-	<i>Escherichia coli</i>	
7.	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus spp</i>	
8.	Irregular colorless flat	+	Short rod	Singles	+	-	+	-	-	-	-	+	+	A	A	-	<i>Bacillus spp</i>	
9.	Irregular Milky Raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	A	A	+	<i>Micrococcus spp</i>	
10.	Round Red Raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	A/G	A/G	-	<i>Klebsiella spp</i>	
11.	Circular white convex	+	Cocci	Cluster	+	-	-	-	-	-	+	+	-	A	A	-	<i>Lactobacillus spp</i>	
12.	Circular white convex	+	Cocci	Cluster	+	-	-	-	-	-	+	+	-	A	A	-	<i>Lactobacillus spp</i>	
13.	Circular white convex	+	Cocci	Cluster	+	-	-	-	-	-	+	+	-	A	A	-	<i>Lactobacillus spp</i>	
14.	Round red raised	-	Rod	Cluster	+	+	+	+	-	-	-	+	+	A/G	A/G	-	<i>Klebsiella spp</i>	
15.	Irregular milky flat	-	Rod	Pairs	+	+	-	+	-	-	+	+	+	A	A	-	<i>Escherichia coli</i>	
16.	Circular white convex	+	Cocci	Clusters	+	-	-	-	-	-	+	+	-	A	A	-	<i>Lactobacillus spp</i>	
17.	Milky irregular edge	+	Rod	Singles	+	-	-	-	-	-	+	+	+	A	A	-	<i>Bacillus spp</i>	
18.	Pink round irregular edges	-	Rod	Clusters	+	-	-	+	-	+	+	+	-	A	A	+	<i>Salmonella spp</i>	
19.	Irregular milky raised	+	Cocci	Singles	+	-	-	-	-	-	+	+	+	A	A	+	<i>Micrococcus spp</i>	
20.	Circular yellowish convex	+	Cocci	Chains	+	-	+	+	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus spp</i>	

Comment [FJAG15]: Suspected or identified organism? This wording is synonymous with imprecision

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21.	Circular yellowish convex	+	cocci	Chains	+	-	+	+	-	-	-	-	+	+	A/A	A/A	-	<i>Staphylococcus spp</i>
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The total fungal screening (Table 5) revealed that *Aspergillus* species, *Mucor* species, *Rhizopus* species etc. were the fungal isolate obtained.

**Table 5: Fungal characterization and identification**

Macroscopic features	Microscopic features	Suspected organisms
White to dark grey surface	Non – septate	<i>Mucor</i> species
Black and white pigments	Septate	<i>Rhizopus</i> species
Wooly white with orange spots	Non – septate	<i>Rhizopus</i> species
Wooly white turned black	Septate	<i>Aspergillus</i> species
Cottony white irregular shape greenish-yellow	Septate	<i>Aspergillus</i> species

**Comment [FJAG16]:** I suggest considering features with taxonomic value. The colors of the mycelium are not entirely reliable.

### Discussion

The results obtained showed that soil samples impacted with palm oil mill effluents (POME) yielded low microbial population and diversity when compared to soil samples without palm oil mill effluents. This is in disagreement with work of Iyakndue *et al.* (2017) who opined that there was an increase of fungal isolates in soil impacted with palm oil mill effluents which could be due to the fact that fungal isolates thrive well in acidic environment. It was also observed that as the POME site gets older, microbial numbers and diversity in the affected soils increase. This finding may not be so surprising because palm oil mill effluent at the time it was being discharged into the receiving soil is often very hot (Orji *et al.*, 2006).

**Comment [FJAG17]:** Point out that microbes

The hot effluent may have affected the soil microflora as the nearby soils which were used as control did not show such drop in microbial number and

diversities. This agrees with the work of Tubonimiet *al.*, (2007) who stated that the continual applications of effluents on the soil can change soil properties, examples pH and nutrient concentrations. Acclimatization and adaptation could therefore be one of the reasons for the isolation of more microorganisms in soils with older POME deposit. The findings also showed that soils with older POME deposit tend to have more numbers and varied microorganisms than nearby soils without POME deposit. This may be because palm oil mill effluents (POMES) are rich in biodegradable organic matter, and as the microorganisms biodegrade the organic content of the POME, the receiving soil inevitably is also influenced. The organic nature of palm oil mill effluent (POME) indicates that the effluent is biologically active and degradable. This corroborates the findings of Hemming, 2000 and Sinnapa, 2001.

The biochemical characterization and identification revealed the presence of the following suspected organisms; *Klebsiella spp.*, *Staphylococcus spp.*, *Bacillus spp.*, *Aspergillus spp.*, *Rhizopus spp.*, *Mucor spp.* This observation is in agreement with the works of Nigeria Institute for Oil Palm Research (NIFOR), Osaro (2002), who found that the genera *Pseudomonas sp.*, *Bacillus sp.*, *Penicillium sp.* and *Aspergillus sp.* were predominant. Rajagopalan (2000) and Sinnapa (2000) showed that the variation in organisms from the mill effluent could be attributed to the nature of the environment, whether the microorganisms are mesophilic or thermophilic and that the population changes along the disposal channel. This is in line with the observations of Osaro (2002) who noted that the nature and behaviour of the microbial population in the POME environment are influenced by many physio-chemical parameters of ecological importance. The presence of coliforms in this study indicates the presence of faecal contaminants. This result implies that palm oil mill effluents have inhibitory properties as it

**Comment [FJAG18]:** The taxonomic characterization revealed the presence of the following suspected organisms?

poses great threat to soil microorganisms which in turns affect soil fertility and crop yields.

## **CONCLUSION**

The analysis on the effect of palm oil mill effluent on soil microbial communities showed that palm oil mill effluent had great effects on soil microorganisms, and could pose a great threat if not properly managed. This is because the continuous application of palm oil mill effluent on soil can lead to changes that affect soil microbiota and consequential soil fertility. Leading to low farm produce when discarded on to farm lands. Therefore, government should develop a means of controlling these illegal discharges by creating awareness on the negative implications.

## **REFERENCES**

1. Adedokun, O. M and Ataga, A. E. Effects of amendments and Bioaugmentation of soil polluted with Crude Oil, automotive Gasoline Oil, and spent engine oil on the growth of cowpea (*Vigna unguiculata* L Walp). Scientific Research and Essay. 2007. 2 (5): 147-149.
2. Adenipekun, C. O. Bioremediation of Engine Oil polluted soil by *Pleurotus Tuber-regium* Singer, a Nigerian White-rot Fungus. African Journal of Biotechnology. 2008. 7(1): 055058.
3. Ahmad A., Ismail S., and Bhatia S. Water recycling from palm oil mill effluent (POME) using membrane technology. Desalination. 2003. 157:8795
4. Cheesbrough, M. District laboratory practice in tropical countries. 2002. 55: 214-217.
5. Hartley, C. W. S. Environmental Impact of Oil Palm Plantations in Malaysia. Palm Oil Research Institute of Malaysia (PORIM) Occasional Paper. 2004. 33: 1-27.

6. Izah, S. C. and Ohimain, E. I. Microbiological quality of palm oil used in Nigeria: Health impacts perspective. *Point Journal of Botany and Microbiology Research*. 2016. 2(1): 037 – 045.
7. Iyakndue, M.L, Brooks, A.A., Unike, A.A., and Agbo, B.E. Effect of palm oil mill effluent on soil microflora and fertility in Calabar. *Asian journal of Biology*. 2017. 2(3): 1-11.
8. Kolwzan, B., W. Adamiak, K. Grabas and Pawleczyk, A. *Introduction to Environmental Microbiology*. Oficyna Wydawnicza Politechniki Wrocławskiej, Wrocław. 2006.
9. Li, H., Y. Zhang, C. G. Zhang and Chen, G. X. (2005). Effects of Petroleumcontaminating waste water irrigation on bacterial diversity and enzymatic activities in a paddy soil irrigation area. *Journal of Environmental Quality*. 2005. 34: 1073-1080
10. Mohammed, R.R., and Chong, M.F. Treatment and decolorization of biologically treated Palm Oil Mill Effluent (POME) using banana peel as novel biosorbent. *Journal of Environmental Management*. 2014. 132, 237–249.
11. Ogboghodo, I. A., Osemewota, I.O., Eke, S.O. and Iribhogbe, A. E. Effects of Cassava (*Manihot esculenta* Crantz) grating mill effluent on textural, chemical and biological properties of surrounding soil. *World Journal of Biotechnology*. 2001. 2: 292-301.
12. Olorunfemi, D., E. O. Emoefe, and Okieimen, F. E. Effect of cassava processing effluent on seedling height, biomass and chlorophyll content of some cereals. *Research Journal of Environmental Sciences*. 2008. 2 (3): 221-227.
13. Onuh, M. O., O. K. Maduikwe, and Ohia, G. U. Effects of poultry manure and cow dung on the physical and chemical properties of crude oil polluted soil. *ScienceWorldJournal*. 2008. 3: 45-50.
14. Orji, M. U., Nwokolo, S.O. and Okoli, I. Effects of palm oil mill effluent on soil Microflora. *Nigerian Journal of Microbiology*. 2006. 20(2): 1026-1031.
15. Tubonimi, J.K., Patricia, U.A., Herbert, O.S. and Amonia, O.B. Impact of palm oil mill effluent on water quality. *Reserve journal of applied science*. 2007. 2(7):842-845.
16. Willey, J. M, L. M. Sherwood and Woolverton, C.J. Prescott, Harley, and Klein's *Microbiology* 7th Edition. McGraw -Hill, New York, 2008. 1-1088.