

## Effluent from local palm oil mill refinery in Nigeria is excessively oily and potentially genotoxic

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### Abstract

In spite of the fact that the informal, local mills in countries like Nigeria constitute a major portion of the palm oil refinery, adequate attention has not been paid to the quality of the palm oil mill effluent (POME) they generate. In this study, therefore, the physicochemical properties and genotoxic potential of POME generated by a local mill in Nigeria were investigated using the methods prescribed by the American Public Health Association and *Allium cepa* chromosomal assay, respectively. In addition to the presence of trace/toxic metals (Zn, Pb, Cd, Cr, Cu) and high biochemical oxygen demand, POME contained a very high oil and grease concentration of 10,500 mg L<sup>-1</sup> as against the maximum limit of 25 mg L<sup>-1</sup> prescribed in the Environmental Management Guideline for the Palm Oil Industry. Mitotic activities in *A. cepa* roots exposed to POME showed that the number of dividing cells and percentage mitotic index generally decreased with increasing POME concentrations. The major chromosomal aberrations induced by POME were sticky, C-mitosis, bridged anaphase, vagrant, and attached chromosomes. However, no chromosomal aberrations were observed in onion roots exposed to water (control). These results indicate that the local refinery from where the POME was obtained is inefficient at recovering oil from palm fibre. The effluent generated by the mill is also a potential pollutant capable of inducing genotoxic and other adverse effects. These results may be typical of many more local palm oil refineries who use mainly manual methods to extract oil from oil palm fruits.

**Keywords:** chromosomes; cytotoxicity; mitosis; pollutants; POME

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### Introduction

The oil palm industry is a major agro-based business in Africa, especially West Africa where oil palm trees are found both in the wild and in plantations (Eze *et al.*, 2013). In Nigeria, there are a number of corporate palm oil refineries, but the business of palm oil extraction is dominated by the local, small scale and peasant farmers (Orji *et al.*, 2006) who use mainly manual methods to extract oil from oil palm fruits.

Palm oil processing generates a number of waste by-products, such as empty fruit bunches, oil palm shell, palm fibre and palm oil mill effluent (POME) (Rupani *et al.*, 2010; Otti *et al.*, 2014). Of these wastes, POME

is considered the most environmentally harmful, when discharged untreated (TDIW, 1997; Rupani *et al.*, 2010; Madaki and Seng, 2013). POME is the liquid waste that comes from the sterilization and clarification sections of the oil palm milling process. It is composed of 4-5% solids (mainly organic), 0.5-1% residual oil, and about 95% water containing high concentration of organic nitrogen (Onyia *et al.*, 2001). The processing stages involved in the extraction of oil from oil palm fruit in corporate and local mills are similar to a large extent. However, local milling of palm oil is relatively longer and laborious.

In local milling, palm fruit bunches are quartered (cut into four) and left overnight for easy separation of nuts from the spikelet. The fruits are boiled for about 1-2 hours, pounded in a mortar or macerated with the feet in a container. Water is added into the boiled, macerated fruits, and the mixture is properly mixed together. All the nuts are thereafter carefully removed from the mixture. The fibres are then thoroughly and carefully shaken over in the sludge, until oily foam comes up to the surface of the sludge. The oily foam is carefully collected from the surface into a container until the operation is completed, when there is no more foam formation. The foam is later boiled for about 30 to 40 minutes. Clean edible oil is collected on the surface leaving the sludge at the bottom. Sometimes the remnant oil in the sludge pit is recovered and mixed with fibre to make a fire-starting cake. The sludge that is eventually left is the POME. The resulting POME is recycled, or poured onto the surrounding water, bush, or soil, depending on the location of the refinery.

While corporate mills use large volumes of water and generate correspondingly large volumes of POME, local, small-scale mills release relatively less volumes of POME, because the POME generated is often reused, probably to recover more oil (Ohimain *et al.*, 2012), or possibly due to limited water availability. Most times, when POME generated from the local mills are discarded, they are discharged, with little or no treatment (Okwute and Isu, 2007).

Many research works have evaluated the environmental burdens created by POME (Okwute and Isu, 2007; Igwe and Onyeagbado, 2007; Rupani *et al.*, 2010; Ohimain *et al.*, 2012; Syrat *et al.*, 2014; Dada *et al.*, 2018). Most of these studies are however centred on the observed harmful physico-chemical properties of POME generated by corporate palm oil refineries. This is in spite of the fact that the informal, local, small-scale mills in countries like Nigeria constitute a major portion of the palm oil refinery. This study therefore aimed to assess the physicochemical properties and genotoxic potential of POME obtained from a local mill in Nigeria.

## Materials and Methods

### *Collection of test samples*

The POME used for this study was obtained from a local, small-scale refinery in South Western State of Nigeria (Figures 1 and 2). POME was collected in a 10-litre high density plastic container and transported to the laboratory, where it was refrigerated at about 4 °C during the period of the study. The *A. cepa* (onions/onion bulbs) used for the study were procured from Bariga market in Lagos, Nigeria. The onions were sun-dried for two weeks, after which the dry ones were selected for the test.

### *Physicochemical analyses of POME*

POME samples were analysed for physicochemical properties (pH, DO, BOD, COD, suspended solids, total nitrogen, oil and grease, metals (Pb, Zn, Cd, Cu, Cr) using the methods prescribed by the American Public Health Association (APHA) (1998). Some results of the physicochemical analyses were compared with the limits contained in the Environmental Management Guideline for the Palm Oil Industry published by the Thailand Department of Industrial Works (TDIW, 1997); this was the only readily available guideline specifically released for the palm oil industry.



**Figure 1.** Processing of palm fruit in the local refinery from where POME was collected



**Figure 2.** Ground-dug refining sludge pit from where POME was collected

To analyse the metals, 100 mL of POME sample was digested by heating with concentrated  $\text{HNO}_3$  until the volume was reduced to 3-5 mL. This volume was made up to 10 mL with 0.1 N  $\text{HNO}_3$ . The concentration of the metal was estimated by using an Atomic Absorption Spectrophotometer (Perkin Elmer Analyte A 200 model).

#### *Procedure for A. cepa assay*

The *Allium* test was adapted from Fiskesjo (1997), Adegbite and Olorode (2002), Dada *et al.* (2018). The outer scales and brownish bottom plates of sun-dried onion bulbs were carefully removed, leaving the ring of primordial root intact. The peeled bulbs were placed in de-chlorinated tap water during the cleaning procedure to prevent the primordia from drying up. Graduated concentrations of POME (10%, 20%, 30%, 100%) were prepared by diluting with the appropriate volumes of de-chlorinated water. The peeled onion bulbs were first grown in de-chlorinated tap water at room temperature for 24 hours. After 24 hours, the bulbs were transferred to the prepared concentrations of POME (10%, 20%, 30%, 100%). The onions grown in water only served as the control. The test substrates were changed daily throughout the test period. Six onion bulbs were set up for each concentration, out of which the best five were selected for evaluation.

To determine root growth inhibition by POME, the root lengths of the onions exposed to each POME concentration, and the ones exposed to water (control) were measured at 24, 48, and 72 hours of exposure. Mean root length, percentage root length, and percentage root length inhibition were calculated according to the following Equations 1, 2, and 3, respectively.

$$\text{Mean root length (cm)} = \frac{\text{Summation of root lengths}}{\text{Total number of roots}} \quad (1)$$

$$\text{Percentage root length} = \frac{\text{mean root length in test solution}}{\text{mean root length in control}} \times 100 \quad (2)$$

$$\text{Percentage (\%) root length inhibition} = \frac{\text{mean root length in control} - \text{mean root length in test solution}}{\text{mean root length in control}} \times 100 \quad (3)$$

#### *Cytological assessment*

Chromosomal aberration assessment was carried out by the squash technique for onion root as described by Adegbite and Olorode (2002). At the end of the exposure periods, the roots of onion bulbs with the best growth at each concentration were removed with forceps and fixed in 1:3 aceto-alcohol. Cells for cytological studies were prepared from the root tip meristem containing actively growing cells. One root tip was squashed on each slide, and stained with acetocarmine for 10 minutes. Cover slips were carefully lowered onto the slide, to exclude air bubbles. To prevent the possible drying out of the preparation, the cover slips were sealed onto the slides with clear fingernail polish. Each prepared slide was viewed under the 40x objective lenses of the light microscope (Leica 2000 phase contrast microscope) to observe its mitotic stages. Mitotic index and percentage mitotic index were calculated according to Equations 4 and 5, respectively.

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells counted}} \times 100 \quad (4)$$

$$\text{Percentage Mitotic Index} = \frac{\text{Mitotic index in control} - \text{mitotic index in test solution}}{\text{Mitotic index in control}} \times 100 \quad (5)$$

Scoring of chromosomal aberrations was taken from 5 microscopic fields for each of the POME concentrations. Percentage chromosomal aberration was calculated according to Equation 6 below.

$$\text{Percentage aberration} = \frac{\text{Total number of chromosomal aberrations}}{\text{Total number of dividing cells}} \times 100 \quad (6)$$

#### *Statistical analysis*

The data generated were analysed using one-way analysis of variance (ANOVA). Mean differences were compared for level of significance using the Least Significant Difference (LSD) post hoc. All statistical analyses were carried out using the Statistical Package for Social Sciences (version 22.0).

## **Results**

### *Physicochemical characteristics of POME*

The physicochemical parameters of POME are presented in Table 1, along with the limits set in the Environmental Management Guideline for the Palm Oil Industry, published by TDIW. The pH of the POME was 5.9 while the turbidity, alkalinity, and acidity levels were 491.0 NTU, 296.0 mg L<sup>-1</sup>, and 349.2 mg L<sup>-1</sup>, respectively. The concentrations of phosphate and potassium in the POME were 198.60 mg L<sup>-1</sup> and 2119.51 mg L<sup>-1</sup>, respectively. The total nitrogen in the POME was 711.95 mg L<sup>-1</sup> while the oil and grease content were

10,500.00 mg L<sup>-1</sup>. The concentrations of Pb, Cu, Zn, and Cd in POME were 0.04 mg L<sup>-1</sup>, 0.66 mg L<sup>-1</sup>, 2.89 mg L<sup>-1</sup>, and 0.01 mg L<sup>-1</sup>, respectively.

**Table 1.** Physicochemical properties of POME

Qualities	Concentrations	
	POME	TDIW limits
<b>Physical</b>		
pH	5.9	5.5-9.0
Turbidity (NTU)	491.0	<1,000
Electrical conductivity $\mu\text{S cm}^{-3}$	2,568.0	Na
Total dissolved solids mg L <sup>-1</sup>	1,284.0	Na
<b>Chemical</b>		
Oil and grease mg L <sup>-1</sup>	10,500.0	<25
Total alkalinity mg L <sup>-1</sup>	296.00	Na
Total acidity mg L <sup>-1</sup>	349.2	Na
Biological oxygen demand (BOD) mg L <sup>-1</sup>	125.0	<100
Chemical oxygen demand (COD) mg L <sup>-1</sup>	1,786.0	<1000
Dissolved Oxygen (DO) mg L <sup>-1</sup>	3.9	Na
Chloride mg L <sup>-1</sup>	441.0	Na
Total nitrogen mg L <sup>-1</sup>	711.95	<50
Potassium mg L <sup>-1</sup>	2,119.51	Na
Phosphate mg L <sup>-1</sup>	198.60	Na
<b>Metals</b>		
Zinc mg L <sup>-1</sup>	2.89	Na
Lead mg L <sup>-1</sup>	0.04	Na
Cadmium mg L <sup>-1</sup>	0.01	Na
Chromium mg L <sup>-1</sup>	0.18	Na
Copper mg L <sup>-1</sup>	0.66	Na

POME = Palm oil mill effluent; TDIW = Thailand Department of Industrial Works (1997); Na = not available.

#### *Effect of POME on the root growth of A. cepa*

POME produced inhibitory effects on the root growth of *A. cepa* at 20%, 30%, and 100% concentrations, but promoted root growth at 10% concentration. The highest inhibitory effects were produced at 72 hours of exposure while the lowest inhibitory effects were produced at 24 hours of exposure. Generally, root growth inhibitory effects increased with rising concentrations, especially at 48 and 72 hours of exposure. POME of 30% concentration produced the highest root growth inhibitory effects of 55.09% at 72-hour of exposure. On the other hand, POME of 10% concentration promoted root growth to the tune of 7.23% at 24-hour of exposure (Table 2).

#### *Mitotic activity in A. cepa exposed to different concentrations of POME*

The mitotic activities in *A. cepa* roots exposed to POME showed that the numbers of dividing cells and percentage mitotic index generally decreased with increasing POME concentrations. In most cases, especially at 24-hour and 72-hour of exposure, the percentage mitotic inhibition increased with rising POME concentrations. The highest mitotic inhibition of 78.28% was induced by 100% POME concentration, at 72-hour of exposure. The least mitotic inhibition of 57.96% was also induced by 100% POME concentration, but at 48-hour of exposure (Table 3).



**Table 2.** Effect of POME on the root growth of *A. cepa*

PC	Mean root growth (cm)			% Root growth			% Root growth inhibition		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
<b>Ctr (0%)</b>	0.83±0.30	1.65±0.35	1.67±0.38	100.00	100.00	100.00	00.00	00.00	00.00
<b>10%</b>	0.89±0.30	1.29±0.45	1.29±0.23	107.23	78.18	77.25	-7.23#	21.82	22.75
<b>20%</b>	0.79±0.16	0.80±0.25	0.80±0.19	95.18	48.48	47.90	4.82	51.52	52.10
<b>30%</b>	0.70±0.24	0.75±0.07	0.75±0.16	84.34	45.45	44.91	15.66	54.55	55.09
<b>100%</b>	0.77±0.12	0.77±0.21	0.77±0.28	92.77	46.67	45.29	7.23	52.69	53.89

PC = POME concentration; ctr = control; hrs = hours; # = root growth not inhibited but promoted by 7.23%, relative to the control.

**Table 3.** Mitotic activity in *A. cepa* exposed to different concentrations of POME

PC	Total cells counted			Number of dividing cells			% Mitotic index			% Mitotic inhibition		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
<b>Ctr (0%)</b>	500	500	500	126	98	90	25.20	19.60	18.00	00.00	00.00	00.00
<b>10%</b>	485	473	469	43	22	31	8.87	4.65	6.61	64.80	76.28	63.28
<b>20%</b>	481	468	464	39	27	26	8.11	5.77	5.60	67.82	70.56	68.87
<b>30%</b>	476	463	459	33	32	21	6.93	6.91	4.58	72.50	64.75	74.56
<b>100%</b>	465	449	435	27	37	17	5.81	8.24	3.91	76.94	57.96	78.28

PC = POME concentration; ctr = control; hrs = hours

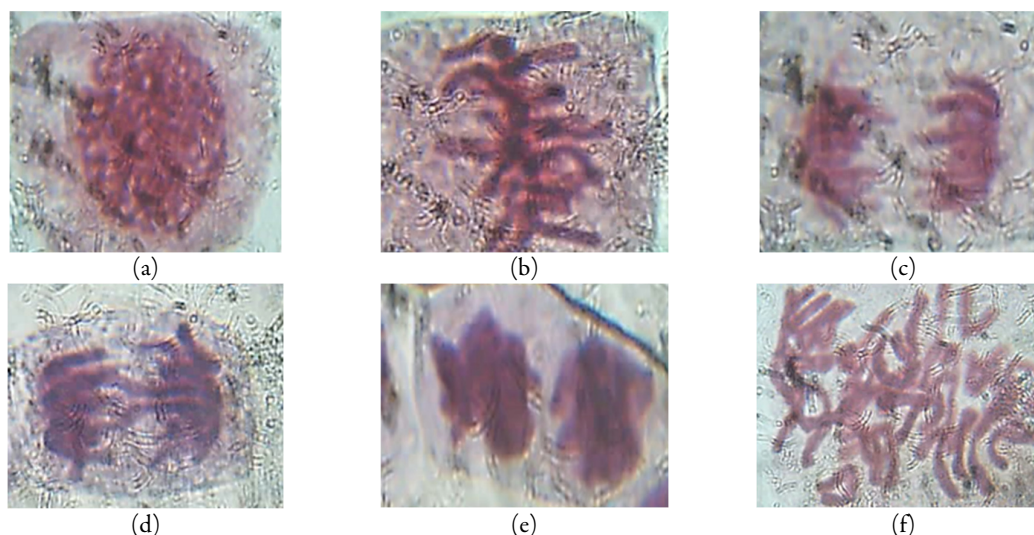
#### *Chromosomal aberrations induced by POME*

POME induced chromosomal aberrations in *A. cepa* roots, but no aberration was observed in *A. cepa* exposed to water (control). The major aberrations induced were sticky, C-mitosis, bridged anaphase, vagrant, and attached chromosomes. Sticky and bridged chromosomes were the most frequent, recording a total of 78 and 52, respectively. Onions exposed to 10% POME concentration recorded the highest total aberration and highest percentage aberration of 61 and 63.54%, respectively. The lowest percentage aberration of 45.80% was recorded by onions exposed to POME of 30% concentration (Table 4). Figure 3 shows representative normal and aberrant dividing cells.

**Table 4.** Major chromosomal aberrations induced in *A. cepa* root cells exposed to different concentrations of POME

PC	Chromosomal aberration type																Total Abr	% Abr
	Sticky			C-mitosis			Bridged anaphase			Vagrant			Attached					
	24 hrs	48 Hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr			
10%	8	7	9	2	2	0	6	5	5	4	4	3	2	2	2	61	63.54	
20%	9	6	6	1	0	0	6	4	3	4	4	2	2	3	3	53	57.61	
30%	7	7	5	3	0	0	5	4	3	3	3	2	3	2	2	49	45.80	
100%	5	5	4	0	1	0	4	4	3	3	2	4	2	2	1	40	49.38	
Total	Sticky 78			C-mitosis 9			Bridged 52			Vagrant 38			Attached 26			203		

PC = POME concentration; Abr = aberration; hr = hour; the differences in total aberrations induced by different POME concentrations are not significant ( $p > 0.05$ ).



**Figure 3.** Photomicrographs of some representative normal and aberrant dividing cells  
 a = normal prophase; b = normal metaphase; c = normal anaphase; d = bridged chromosomes; e = sticky chromosomes;  
 f = vagrant chromosomes.

## Discussion

Physicochemical analyses, including heavy metal characterization, are a common method of assessing toxicity. In this study, many physicochemical parameters of POME, including electrical conductivity, total dissolved solids, BOD, COD, potassium levels were high, and in some cases, far higher than the limits contained in the Environmental Management Guideline for the Palm Oil Industry, released by TDIW (1997). Moreover, when compared with previous studies (Ohimain *et al.*, 2012; Dada *et al.*, 2018), most of the physicochemical parameters were relatively higher. A particular instance is the oil and grease concentration which was over 4,000 folds higher than the limit of 25 mg L<sup>-1</sup> set by TDIW. A major implication of the very high oil and grease concentration is that local palm oil milling is inefficient at recovering oil from palm fibre. With such high concentrations of oil and grease, and other polluting parameters in POME, it may be assumed that local palm oil milling is potentially unsustainable, as it constitutes a threat to the health of the environment.

When oil and grease polluted effluent, like the POME evaluated in this study, is discharged into a water body, it may create health issues and lead to emission of odour due to delayed eutrophication. In addition, the oil and grease components of a wastewater can form a layer on the surface of water, leading to reduced DO and increased COD and BOD, all of which disrupt ecosystem balance, and threaten aquatic life. Oil and grease-laden wastewater may also be unsuitable for farm irrigation as it can block irrigation systems (Sahu *et al.*, 2007; Travis *et al.*, 2008; Khwakaram, 2016). If discharged on land, oil and grease polluted wastewater can block soil pores, leading to anaerobic conditions. Soil anaerobic condition can in turn lead to unpleasant odour, inhibited plant growth, and degraded soil quality (Travis *et al.*, 2008). High oil and grease concentration decreases the effectiveness and efficiency of wastewater treatment processes (McPhail, 2015).

The low mitotic index, high mitotic inhibition, and aberrant chromosomes observed in this study are indications that the POME generated by the local mill is mitodepressive, cytotoxic, and genotoxic. These effects likely resulted from the high pollutant loads in the POME. Mitotic index is considered a reliable assessment of the presence of cytotoxic pollutants in the environment (Kaymak and Goc-Rasgele, 2009; Radic *et al.*, 2010). The low mitotic index and high mitotic inhibition were likely brought about by the suppression or disruption of DNA replication by pollutants, such as metals, contained in POME (Sudhakar *et al.*, 2001). Metals are capable of inducing decreased mitotic index by interacting with DNA to bring about cell cycle disruption and

chromatin dysfunction (Glinska *et al.*, 2007). Sticky chromosome, which was the most frequently observed aberration, results from chromatin dysfunction, chromatid breaks, and spindle failure, which suggests the presence of clastogens (agents capable of inducing chromosome/chromatid breaks) and aneugens (agents capable of inducing chromosome/chromatid gain or loss) in the effluent (Leme and Marin-Morales, 2009). Sticky chromosomes lead to abnormal uncoiling of chromosomes between anaphase and telophase (Qian *et al.*, 2006). Sticky chromosome signifies high toxicity that could result in cell death. Bridged chromosomes could result from the general stickiness of the chromosomes at metaphase stage, or breakage and reunion of chromosomes (Chandel and Tank, 2016).

In this study, POME promoted root growth at 10% and yet induced the highest chromosomal aberrations at that same concentration. This probably implies that a particular wastewater may promote growth and still be genotoxic. Therefore, it is advisable that any wastewater that will be considered for farm irrigation should be tested for genotoxicity, even after treatments.

In a related study (Dada *et al.*, 2018), POME generated by a corporate mill also had similar mitodepressive, cytotoxic, and genotoxic effects on the roots of *A. cepa*, but generally to a lower extent. Though, POME is inherently an environmental pollutant, the relatively higher pollutant loads in the present study, especially oil and grease, could be partly due to the fact that local palm oil millers do not subject their POME to any form of treatment (Okwute and Isu, 2007). Moreover, some of them do recycle POME which ought to be disposed of (Ohimain *et al.*, 2012). These practices increase the tendency of local mill-generated POME to constitute pollution threat to the environment, ecological systems, and biodiversity. Since local milling generates a substantial quantity of palm oil consumed in Nigeria, and is a source of income and livelihood to many rural and town dwellers, their operations cannot be easily dispensed with. Therefore, for the safety of the environment, ecological systems, and biodiversity, it is imperative for the Nigerian governments and stakeholders to reach out to local millers with a view to discouraging them from reusing POME for palm oil milling. Reusing or recycling POME potentially increases retention of pollutants, including oil and grease. POME is naturally biodegradable, but the process is too slow to cope with the volumes generated. As such, local millers should also be encouraged to limit their operations to locations where there will be adequate spaces for the POME they generate to undergo natural biological degradation.

## Conclusions

POME generated by a local mill was assessed in this study. The POME contained a very high concentration of oil and grease, in addition to other pollutants. The POME was also mitodepressive and cytotoxic, inducing various types of chromosomal aberrations. The observed low mitotic index, high mitotic inhibition, and aberrant chromosomes in the root cells of *A. cepa* likely resulted from the high pollutant loads in the POME. Although, the test was conducted on POME from one local mill, the results may be typical of many more local palm oil refineries who use mainly manual methods to extract oil from oil palm fruits. Local millers should be discouraged from reusing or recycling POME, and encouraged to limit their operations to locations where there will be adequate spaces for the POME they generate to undergo natural biological degradation.

## Authors' Contributions

EOD designed the research and wrote the draft manuscript. OMG collected research samples and carried out laboratory analysis on the samples. MOS interpreted chromosomal data and took part in manuscript writing. HOI and OOA carried out statistical analysis and interpreted the data.

All authors read and approved the final manuscript.



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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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