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Microbial Evaluation of Palm Oil Mill Effluent Impacted Environment in Rivers State, Nigeria

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ABSTRACT

Palm oil mill effluent (POME) is a high-strength organic waste produced during the processing of palm oil and is discharged untreated into the surrounding environment. This study determined the microbiological and physicochemical parameters of POME-impacted environments in Rivers State, Nigeria as to evaluate the impact of the effluents on the microbial diversity and quality of the surrounding environment. Standard techniques were employed for the collection of soil, water and air samples from Elele and Erema locations and to enumerate, isolate and identify the microbial populations and for the determination of physicochemical parameters. Results of total heterotrophic bacterial (THB) count for dry season ranged from 0.48±0.4 to 2.4±0.6×10^7 CFU/g, while the fungal counts ranged from 0.40±0.3 to 4.6±1.6×10^4 SFU/g. The THB of POME impacted soil in Elele location was significantly ($P<0.05$) higher than only the Elele control, while the fungal count of the POME impacted soil in Erema was significantly ($P<0.05$) higher than all other samples including the controls. The THB of samples in raining season ranged from 5.5±1.1 to 21.9±0.9×10^6 CFU/g, while the fungal counts ranged from 0.35±0.2 to 1.7±1.1×10^4 SFU/g. The THB of the POME impacted soil in Elele location was significantly ($P<0.05$) higher than the other samples including the control. *Bacillus* sp, *Enterobacter* sp, *Providencia* sp, *Shigella* sp, and *Micrococcus* sp., were bacteria isolated from the POME-impacted environments. *Rhizopus* sp, *Aspergillus* sp., *Aspergillus flavus*, *Penicillium* sp., and *Fusarium* sp. were associated with POME-impacted environments. The pH of the pond during dry and raining seasons was 4.74 and 7.55 respectively. Soil-parameter ranges were: pH 4.26-5.74, temperature 24.6-32.5°C, EC: 37.06-69.24µS/cm, nitrate: 51.03-74.18mg/kg, phosphate: 13.48-19.67mg/kg and potassium: 103.17-183.49mg/kg. The presence of isolated microorganisms could imply their ability to thrive in nutrient-rich environment associated with POME. Additionally, *Staphylococcus*, *Shigella* and *Enterococcus* sp could be potential pathogens among these isolates which could raise public health concerns. Treatment of POME before discharge and remediation of POME-impacted soil is recommended.

Keywords: Palm oil mill effluent, microbial evaluation, environmental impact, *Shigella*, *Aspergillus*, potential pathogens.

Introduction

Palm oil production is a major industry in many tropical countries, with Malaysia and Indonesia being the largest producers globally (Corley & Tinker, 2016). Due to its economic significance, the cultivation of oil palm has grown quickly (Krungsri Research, 2016). In Nigeria, dispersed smallholder farmers produce 80% of the country's palm oil, which accounts for 40% of the world's vegetable oil production (Solidaridad, 2020). In Nigeria, smallholder farmers manage and plant 1–5 hectares of oil palm (Daemeter Consulting, 2015), making them significant players in the industry (Solidaridad, 2020). These farmers actively participate in the value chain and different production processes. The extraction of palm oil from the fruit mesocarp generates a significant byproduct known as Palm Oil Mill Effluent (POME). POME is a brownish colloidal suspension composed of water, oil, and various solid materials (Ahmad *et al*., 2005) and highly polluting wastewater characterized by its complex physicochemical composition (Corley & Tinker, 2016). Which is highly variable, depending on factors such as the palm oil milling process, the quality of fresh fruit bunches, and the treatment methods employed (Yacob *et al*., 2005).

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Palm oil mill effluent (POME) is a byproduct of palm oil extraction and refining processes. It is a highly polluting wastewater that contains a complex mixture of organic matter, suspended solids, and other contaminants. The composition of POME varies, but it typically includes high concentrations of oil and grease, chemical oxygen demand (COD), biological oxygen demand (BOD), and nutrients such as nitrogen and phosphorus (Chin *et al*., 2015). The discharge of POME into the soil impacts its pH, which is a significant factor influencing nutrient availability to plants (Okwute and Isu, 2007). The acidity of POME influences soil pH, which typically falls within the range of 6.5 to 7.5, a range most conducive for plant growth.

The soil is also affected by the leaching of heavy metals and other physicochemical properties of POME. POME elevates the levels of organic carbon, total nitrogen, phosphate, sulfate, phosphorus, sodium, potassium, calcium, magnesium, aluminum, and hydrogen in the soil (Eze *et al*., 2013). While the presence of nutrients, including sodium, phosphorus, and potassium, typically enhances plant growth, an abnormal pH level can disrupt their effectiveness (Okwute and Isu, 2007). The high phosphorus content in POME-contaminated soil can lead to the delayed release of materials because POME biodegrades slowly (Okwute and Isu, 2007).

The increased availability of phosphorus in soil contaminated with POME is due to alterations in pH levels and other soil nutrient factors (Eze *et al*., 2013). While the solids in raw POME serve as a valuable source of organic matter, which enhances soil productivity, this potential benefit is hindered by the abnormal pH of POME-contaminated soil (Okereke, *et al*., 2020). The present study therefore seeks to evaluate the effect of POME on microbial isolates in the impacted soil, air and the pond water in Elele and Erema communities in Rivers State, Nigeria where palm oil is heavily processed.

Materials and Methods

Description of Study Area

The study location is Elele in Emuoha Local Government Area and Erema in Ogba/Egbema/Ndoni Local Government Area both in Rivers State.

Sample Collections

Soil samples contaminated with POME were collected randomly (completely randomized design) at three depths (0-15cm, 15-30cm and 30-45cm) from three points to form a composite in each location in the palm oil mill producing sites. The samples were collected using hand auger and put into a sterile container and transported to the laboratory within 2 hours of sample collection. Samples were taken monthly in raining and dry season from the palm oil mill site. The control involved area not contaminated with the POME in of same location. Water samples were obtained from the pond located at the Erema palm oil mill site using a sterile container. This was stored and transported in an ice-packed container. The soil samples were properly labelled, put in a sterile polyethylene bag and placed in an iced pack container, then transported to the laboratory for analysis.

Physicochemical Properties

The physicochemical properties such as biological oxygen demand (BOD), chemical oxygen demand (COD), turbidity, electrical conductivity (EC), temperature, nitrate, phosphate, and potassium. The American Public Health Association (APHA, 2012), method was used in determining the physicochemical parameters of the water.

Hydrogen Ion Concentration (pH)

The pH was done as described by (APHA, 2012). The pH of the samples was measured using the pH meter model D46 (pH/MV/OC meter). The pH was calibrated using standard buffer solutions of pH 7, 4 and 10. After calibration of the meter, the pH of the sample was analysed by emmersing the pH meter into a 250mL beaker containing 50mL of the water sample. Soil sample(10g) was weighed into 100ml beaker; 50ml of distilled water was then added to allow immersion of the electrode, mixing was carried out by stirring frequently for few minutes. Then beaker was allowed to stand for 15 minutes. The electrode was immersed into the sample. The pH value for each sample was recorded accordingly.

Electrical Conductivity

To determine the electrical conductivity, a standard solution of potassium chloride of known conductivity cell was used (0.01NKCl, 745.6mg in 1.0L de-ionized water = 1413µmhos/cm).

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Thus, the conductivity cell (electrode) was washed three times in the 0.01N KCl solution and the conductivity of the solution measured. The conductivity cell finally immersed into the sample and the conductivity recorded (APHA, 2012).

Biological Oxygen Demand (BOD)

Airtight 300ml capacity BOD bottles were filled to the brim with the samples. The initial dissolved oxygen in the sample was determined. The diluent was prepared by measuring out 22.5g/L $MgSO_4.7H_20$, 27.8g/L $CaCl_2.2H_2O$, and 0.25g/L $FeCl_3$. $6H_2O$, Phosphate buffer: 8.5g $KHPO_4$; 21.7g of K_2HPO_4. $7H_2O$; 1.7g of NaCl and pH 7.2, into a measuring beaker making up the volume to 1L with distilled water. The contents of the flask were mixed by swirling gently and covered. The dilution water was first saturated with dissolved oxygen by shaking in a partially filled bottle before using to dilute the samples. BOD bottles were then filled with the diluted samples and another two bottles with the dilution water to serve as blank. The bottles were stoppered carefully to avoid the entrapment of air. The blank and one experimental BOD bottle were used for the initial dissolved oxygen (DO) determination. The remaining two BOD bottles were water-sealed by filling the flared neck of the bottles with distilled water from a wash bottle. The cover cap supplied with the BOD bottles was used to retain the water. The bottles were incubated at 20^0C for 5 days. At the end of this period, the final DO was determined. The BOD_5in mg/l of the sample was estimated using the formula in equation 1:

$$BOD = DI - \frac{D2}{P} \quad \text{…….. Equation 1}$$

D_1 represents the Dissolved Oxygen (mg/l) of the sample 15 minutes after preparation
D_2 represents Dissolved Oxygen (mg/l) of sample 5 days after incubation at 20^oC
P represents the Decimal volumetric fraction of the sample used, APHA (2012).

Turbidity

This was determined using a standardized Hanna H198703 Turbidometer**.** Distilled water was used to calibrate the Nephelometer (0.0 NTU). Hydrazine sulphate 1.0g was dissolved in 100ml of distilled water to form solution 1.

Also, hexamethylenetetramine 10.0g was dissolved in distilled water and made up to 100ml in a volumetric flask; solution 2. Then 5ml of solutions 1 and 2 was mixed in a volumetric flask and kept for 24 hours at about 25^o C. The mixture was then diluted to 1000ml with distilled water to give a 400 NTU stock suspension. Afterwards, 4ml of the stock solution was diluted to 100ml with distilled water to give 40NTU standard solution. Both mixtures were thoroughly recorded in the Nephelometric tube.

Turbidity (NTU) = Nephelometer readings x Dilution factor ……Equation 2

If turbidity of the sample is >40 NTU, then the sample is diluted, and the dilution factor is accounted for in final calculations (APHA, 2012).

Total Suspended Solids (TSS)

The water sample was used for the total suspended solids' test. Vacuum pump with distilled water was applied to wash the membrane filter (pore size 0.45µm). Suction was done to remove excessive water. The membrane filter was carefully separated, placed in the crucible and dried in the oven at 103 °C for 1 hour. During the analysis, the dried filter paper was wet with a small volume of distilled water and placed in the filtration unit. Fifty milliliters (50ml) of homogenously mixed sample were filtered through the membrane. The membrane filter was carefully removed and transferred to the crucible which contains some content. The content was placed in the oven and dried to constant weight at 103^0C (APHA, 2012).

Total Dissolved Solids (TDS)

This was determined using the Gravimetric method. A portion of water was filtered out and 10ml of the filtrate was measured into a pre-weighed evaporating dish. Following the procedure for the determination of total solids above, the total dissolved solids content of the water was calculated by **s**ubtracting weight of total suspended solids from total solids.

Total dissolved solids (mg) = (W1 – W2) x 1000 ml of the filtrate used …….. Equation 3
L

Where W_1 = initial weight of evaporating dish, W_2 = Final weight of the dish (evaporating dish + residue).

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Nitrate

Nitrate was determined by Phenol Di-Sulphonic acid method by the methods of Jackson (1973) and Trivedy and Goel (1984). Fifty milliliters (50 ml) of the water sample was taken and evaporated over a hot plate till residues were formed, which was dissolved in three milliliters (3ml) of phenol Di-Sulphonic acid. The reaction was allowed to stand for 10 minutes and then fifteen milliliters (15ml) of distilled water was added. Seven milliliters (7ml) of ammonia solution was added and the final volume was made to be fifty milliliters (50ml). The intensity of yellow color transmission percentage was measured at 410 nm. The Values of NO_3-N as mg/l was obtained in reference to the calibration curve and value was computed in the following formula: -

$$Nitrate\ N = \frac{mg\ of\ Nitrate\ N}{ml\ of\ Sample} \quad \text{…….. Equation 4}$$

Phosphate

Phosphorous was estimated as phosphate in the water sample, in four forms viz. Total ortho, Acid hydrolysable, Total and Organic phosphate, following (APHA, 2012). The determination was made by the Vanadomolybdo phosphoric acid method. In this method, ammonium molybdate was reacted under acidic conditions in the presence of vanadium which formed a yellow Vanadomolybdo phosphoric acid. The percentage (%) Transmission of yellow colour was measured at 490nm. The phosphate value was determined with the help of calibration curve prepared from standard solution. The amount of phosphorus/L was calculated by using following formula: -

Phosphate mg/l = mg P x 1000 ml of sample …. Equation 5

Enumeration and Isolation of Microorganisms in POME

Soil samples were prepared by transferring 1g of soil into 10mL sterile normal saline while water samples were prepared by transferring 1mL of the water sample into 9mL sterile normal saline. This was used further diluted using the 10-fold serial dilution to obtain dilutions of 10^{-8}. Aliquots (0.1ml) from a 10^{-2} and 10^{-3} dilution of a ten-fold serial dilution was transferred to freshly prepared nutrient agar and Eosin Methylene Blue agar (EMB) plates in triplicates using a sterile 1ml pipette for the isolation of the total heterotrophic bacteria and total coliform. This was spread evenly with the aid of a flamed glass rod and incubated at 37°C for 48 hours. The total fungal counts were enumerated by inoculating 0.1mL of a 10^{-1} dilution on Sabouraud dextrose agar (SDA) plates supplemented with 100µg of tetracycline. The plates were incubated for 5 days at 25°C. After incubation, colonies that appeared on the plates were counted and the mean expressed as CFU/g for each sample. For air samples, the Koch sedimentation method (Wemedo and Robinson, 2018) was used. Media plates were exposed to the air around the POME sites for 10 minutes. Plates were incubated as stated above.

Isolation of Pure Isolates

Pure bacterial isolates were isolated from the respective plates by picking distinct colonies and streaking on freshly prepared nutrient agar plates. While the colonies on SDA plates were purified by inoculating spores of distinct fungal isolates on SDA plates supplemented with tetracycline antibiotics. The sub-cultured plates were incubated at 37°C for 24 hours for bacteria and 25°C for 2-5 days for fungi. This technique was carried out repeatedly on all isolates until isolates were pure (i.e., void of contaminant).

Identification of Bacterial Isolates

The bacterial isolates were identified based on morphological (appearance on agar plates), microscopical (viewing the Gram stain) and biochemical tests (Wemedo & Robinson, 2018). The Bergey's manual of determinative microbiology (1957) was used to ascertain the identities of the isolates by comparing their morphological and biochemical characteristics with those in the Bergey's manual.

Identification of Fungal Isolates

The fungal isolates were identified based on their macroscopic and microscopic characteristics. The macroscopic characteristics included colony size, texture of the colony, spore colour, while the microscopic characteristics involved the arrangement of hyphae, conidia, or sporangium. The technique described by Robinson *et al*., (2020) was adopted for the microscopic identification of the isolated fungi using lactophenol cotton blue stain.

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In this method, a drop of the stain was placed on clean microscopic slide with the aid of a Pasteur pipette, a small portion of the aerial mycelia from the representative fungi culture was removed and placed in the drop of lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal isolates seen were identified in accordance with standard scheme for identification of fungi as adopted by (Douglas & Robinson, 2018).

Statistical Analysis

The mean and standard deviations of the microbial counts and physicochemical parameters obtained from the analysis was determined using the statistical package for social science (SPSS v27). The analysis of variance was determined to check for significance at 95% confidence interval. The Duncan multiple range test was used in separating the means.

Results

The baseline physicochemical parameters of the palm oil mill effluent impacted pond water during the raining and dry seasons is presented in Table 1. The results showed variations in the physicochemical parameters of the pond in both seasons with that of the WHO permissible limits. The pH of the pond during the dry season was 4.74 (acidic) compared to the near alkaline (7.55) during the raining season. Mores so, the parameters were higher in the dry season than the raining seasons except for the total dissolved solids, turbidity, BOD, potassium and alkaline which were higher in the raining season.

The baseline physicochemical parameters of the palm oil mill effluent impacted soil in Table 2 showed that the pH was all acidic. The pH ranged from 4.26 to 5.74, temperature ranged from 24.6 to 32.5°C, electric conductivity ranged from 37.06 to 69.24 µS/cm, nitrate ranged from 51.03 to 74.18mg/kg, phosphate ranged from 13.48 to 19.67mg/kg and potassium ranged from 103.17 to 183.49mg/kg.

Table 1: Baseline Physicochemical Parameters of POME Impacted Pond Water During Dry and Rainy Season

Parameters	Erema POME (pond) Dry Season	Erema POME (Pond) Raining Season	WHO permissible
pH	4.74	7.55	6.5-8.5
Temperature (^{o}C)	26.30	25.80	26-30
E.C (µS/cm)	840.64	256.40	400
TSS (mg/l)	28.17	28.17	-
TDS (mg/l)	74.26	545.80	500
Turbidity (NTU)	3.50	8.61	5.00
COD (mg/l)	150.57	140.93	20
BOD (mg/l)	28.31	62.74	-
Phosphate (mg/l)	47.52	1.85	-
Potassium (mg/l)	69.46	130.73	12.0
Alkaline (mg/l)	220.57	360.83	250

Table 2: Baseline Physicochemical Parameters for soil samples during dry and raining season

Physicochemical Parameters	Erema cont. soil dry season	Elele cont. soil dry season	Erema cont. soil raining season	Elele cont. soil raining season
pH	4.59	4.26	5.74	5.11
Temperature (^{o}C)	29.80	32.50	24.60	29.10
E.C (µS/cm)	46.11	37.06	58.76	69.24
Nitrate (mg/kg)	74.18	58.58	57.02	51.03
Phosphate (mg/kg)	15.42	19.67	13.48	16.28
Potassium (mg/kg)	128.74	224.25	103.17	183.49

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The microbial counts of the palm oil mill impacted pond water in Table 3 showed that the total heterotrophic bacterial counts (THBC) and total coliform counts were higher ($3.5\pm1.0\times10^6$ and $5.1\pm1.4\times10^5$) in the raining season than in the dry season ($1.51\pm0.9\times10^6$ and $4.0\pm1.1\times10^5$). The fungal counts were higher in the dry season ($3.0\pm1.4\times10^3$) than in the raining season ($2.5\pm0.7\times10^3$. additionally, there were no significant differences (P>0.05) in the bacterial and fungal population of both seasons.

The bacterial and fungal counts of the air samples from the POME environment in Table 4 showed that the Elele POME impacted air had higher bacterial counts (1.1×10^4 CFU/m^3) while the least was from the Erema POME impacted air samples (3.5×10^3 CFU/m^3). The fungal counts of the air samples in the respective POME environment showed that the Elele POME environment had the least ($0.052\pm0.0\times10^3$ CFU/m^3) while the highest was from the uncontaminated samples from the Erema environment ($1.4\pm0.1\times10^3$ CFU/m^3).

Table 3: Microbial Count for Pond water

Season	Total Heterotrophic Bacteria Count ($\times10^6$)	Total Coliform Count ($\times10^5$)	Fungal count ($\times10^3$)
Dry season	1.51 ± 0.9^a	4.0 ± 1.1^a	3.0 ± 1.4^a
Rainy Season	3.5 ± 1.0^a	5.1 ± 1.4^a	2.5 ± 0.7^a
P- value	0.18	0.47	0.69

*Means with similar superscript shared no significant difference (P>0.05)

Table 4: Microbial Load (CFU/m^3) of Air Samples

Stations	Total Heterotrophic Bacteria ($\times10^3$)	Fungal Count ($\times10^3$)
Elele Contaminated	11.5 ± 0.7^b	0.052 ± 0.0^a
Elele Uncontaminated	3.5 ± 0.2^a	1.2 ± 0.4^b
Erema Contaminated	3.5 ± 0.2^a	1.2 ± 0.4^b
Erema Uncontaminated	4.1 ± 0.4^a	1.4 ± 0.1^b
p-value	0.00	0.02

*Means with similar superscript shared no significant difference (P>0.05)

The microbial counts of the POME impacted soil during both the dry season and rainy season in Table 5 showed that in the dry season, the total heterotrophic bacterial count ranged from 0.48 ± 0.4 to $2.4\pm0.6\times10^7$ CFU/g, while the fungal counts ranged from 0.40 ± 0.3 to $4.6\pm1.6\times10^4$ SFU/g. Additionally, the total heterotrophic bacterial counts of POME impacted soil in the Elele location was significantly (P<0.05) higher than the Elele control only, while the fungal count of the POME impacted soil in Erema was significantly (P<0.05) higher than all other samples including the controls. The microbial count of the POME impacted soil for rainy season showed that the total heterotrophic bacterial counts of the samples ranged from 5.5 ± 1.1 to $21.9\pm0.9\times10^6$ CFU/g, while the fungal counts ranged from 0.35 ± 0.2 to $1.7\pm1.1\times10^4$ SFU/g. The total heterotrophic bacterial counts of the POME impacted soil in Elele location was significantly (P<0.05) higher than the other samples including the control.

Additionally, the THB of the POME impacted soil of both locations were higher than their respective controls. While for the fungal counts, there was no significant differences (P>0.05) despite the fluctuations across the samples.

The bacterial isolates from the POME were *Bacillus* sp, *Enterobacter* sp, *Providencia* sp, *Shigella* sp, and *Micrococcus* sp., while for the uncontaminated soil; the bacterial isolates were *Bacillus* sp. *Enterobacter* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Micrococcus* sp, *E. coli*, *Shigella* sp, and *Enterococcus* sp.

The fungal isolates from the POME samples were *Rhizopus* sp, *Aspergillus* sp., *Aspergillus flavus*, *Penicillium* sp., and *Fusarium* sp. while for the uncontaminated sample, *Rhizopus* sp, *Aspergillus* sp., *Penicillium* sp., *Candida* sp and *Fusarium* sp were isolated.

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Table 5: Microbial Count (CFU/g) for Dry Season and Rainy Season of the POME impacted Soil

Stations	Dry Season		Rainy Season	
	Total Heterotrophic Bacteria ($\times 10^7$)	Fungal Count ($\times 10^4$)	Total Heterotrophic Bacteria ($\times 10^6$)	Fungal Count ($\times 10^4$)
Elele Contaminated	2.4±0.6 [b]	1.3±0.6 [a]	21.9±0.9[c]	1.7±1.1 [a]
Elele Uncontaminated	0.48±0.4[a]	0.40±0.3 [a]	4.9±1.0[a]	0.80±0.3 [a]
Erema Contaminated	2.4±0.5 [b]	4.6±1.6 [b]	11.5±2.6[b]	0.35±0.2 [a]
Erema Uncontaminated	1.4±0.7 [ab]	1.3±0.7 [a]	5.5±1.1 [a]	1.7±0.1 [a]
p-value	0.06	0.02	0.01	0.16

*Means with similar superscript shared no significant difference (P>0.05)

Discussion

The findings showed significant environmental impacts associated with palm oil production activities. These findings align with previous research on the subject, highlighting the persistent challenges faced by the industry in managing its environmental footprint. The highly acidic pH of 4.74 observed in the dry season is consistent with findings by Rupani *et al*. (2010), who reported pH values ranging from 4.0 to 5.0 in raw POME. This acidity is attributed to the presence of organic acids produced during the fermentation of palm oil mill wastes.

The seasonal variation in pH, with a more neutral 7.55 in the rainy season, suggests a dilution effect that has been noted in other studies (Awotoye *et al*., 2011). Electrical Conductivity (EC) measurements in this study (840.64 µS/cm in the dry season) exceed those reported by Poh *et al*. (2010), who found EC values ranging from 160 to 260 µS/cm in Malaysian POME.

This discrepancy could be due to differences in processing techniques or local environmental conditions, highlighting the variability in POME characteristics across different production sites. The Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) values observed (150.57 mg/l and 28.31 mg/l respectively in the dry season) are actually lower than those typically reported in previous studies. For instance, Igwe and Onyegbado (2007) reported COD values ranging from 15,000 to 100,000 mg/l and BOD values of 10,000 to 44,000 mg/l in raw POME. This significant difference suggests that the effluent in the current study may have undergone some form of treatment or dilution before reaching the pond, or that the sampling point was not representative of raw POME.

In the dry season, the highest total heterotrophic bacterial count observed in the mill vicinity (4.9 x 10^5 CFU/g), followed closely by the effluent discharge point (4.7 x 10^5 CFU/g) could suggest that the mill's activities contributed to the increased microbial proliferation in the surrounding soil. The higher organic matter content in these areas, likely due to spillage and effluent discharge, provides a rich substrate for bacterial growth (Iwuagwu and Ugwuanyi, 2014). Interestingly, the control site, located 2 km away from the mill, showed the lowest bacterial count (2.8 x 10^5 CFU/g) in the dry season. This observation supports the notion that the mill's influence on microbial populations diminishes with distance from the source of contamination. The rainy season data revealed a general increase in bacterial counts across all sampling points compared to the dry season. This trend can be attributed to the increased moisture content in the soil during the rainy season, which creates more favorable conditions for microbial growth and activity (Fierer and Schimel, 2002). The effluent discharge point exhibited the highest bacterial count (7.2 x 10^5 CFU/g) during the rainy season, significantly higher than other sampling points. This spike in microbial population could be due to the combined effects of increased moisture and the continuous influx of nutrient-rich effluents from the mill. The fungal counts followed a similar pattern to the bacterial counts, with higher populations observed in the rainy season compared to the dry season. The mill vicinity and effluent discharge point consistently showed elevated fungal counts in both seasons, further emphasizing the impact of mill activities on microbial ecology.

The presence of coliforms, *Bacillus*, and *Staphylococcus* species across all sampling points indicates the widespread distribution of these bacterial groups in the soil environment.

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However, the higher occurrence of these microorganisms in the mill vicinity and effluent discharge point, particularly during the rainy season, suggested that palm oil mill activities may be contributing to their proliferation. The detection of *Streptococcus* species solely in the effluent discharge point during the rainy season is noteworthy. *Streptococcus* sp is not typically associated with soil environments and its presence may indicate faecal contamination, possibly from inadequately treated effluents or surface runoff (Byappanahalli *et al*., 2012). This finding raises concerns about potential health risks associated with the discharge of palm oil mill effluents.

Additionally, the findings showed a clear trend of decreasing microbial load with increasing distance from the mill. The highest bacterial and fungal counts were observed at the mill site (0 m), with a sharp decline in counts at 100 m and further reductions at 500 m and 1000 m distances. This pattern of microbial distribution in the air suggests that the palm oil mill serves as a significant source of airborne microorganisms. The high microbial load at the mill site can be attributed to various factors, including the release of bioaerosols during processing activities, the presence of organic matter-rich effluents, and the general industrial operations that can generate and disperse microbial particles (Hameed *et al*., 2012). The observed decrease in microbial load with distance from the mill indicates a gradual dilution effect as the air moves away from the source. However, it's important to note that even at 1000 m distance, the microbial counts remained elevated compared to typical background levels, suggesting a far-reaching impact of the mill's activities on air quality. These findings have significant implications for both environmental and human health. The elevated microbial loads in soil and air around the palm oil mill indicate potential risks of pathogen transmission and allergenic responses, particularly for mill workers and nearby residents. Moreover, the altered microbial ecology in the surrounding environment may have cascading effects on local ecosystems, potentially impacting plant health, soil fertility, and biogeochemical cycles (Tripathi *et al*., 2016).

In conclusion, this study have shown that POME had significant impact in altering the bacterial and fungal population by supporting the proliferation of genera that could tolerate the nutrients and other substances contained in the POME while inhibiting microorganisms that could not utilize the POME. More so, the POME altered the pH and other physicochemical parameters of the soil, air and water. The presence of most of these microorganisms could become potential pathogens especially when contracted by immune-compromised individuals.

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