Original Research Article

Effects of Palm Oil Mill Effluent on Soil Microflora and Fertility in Calabar – Nigeria

Abstract

The study was carried out by artificially polluting an agricultural soil in Calabar with varying concentrations (0ml, 250ml, 500ml, 1000ml and 2000ml) of palm oil mill effluents, with the aim of assessing their effects on soil microflora and fertility. The 0 ml served as a control.The polluted soils were analysed in terms of microbial population, soil pH, organic matter, total nitrogen, available phosphorus, electrical conductivity, calcium, magnesium, potassium, sodium, effective cation exchange capacity, exchangeable acidity, and base saturation. In the polluted soils, the total heterotrophic bacteria, total heterotrophic fungi and total heterotrophic actinomycetes decreased significantly (p<.05) with increase in the concentration of the pollutant. The total heterotrophic bacteria and total heterotrophic fungi showed significant reduction (p<.05) with increase in the duration of the pollution while total heterotrophic actinomycetes showed no significance difference (p>.05) over the duration of pollution. Bacteria isolated from the polluted soils were Pseudomonas sp., Bacillus sp., Staphylococcus sp., Micrococcus sp. Flavobacterium sp., Serratia sp., Proteus sp.Mucor spp., while fungal isolates were Penicillum spp., Aspergillusspp and Rhizopus spp. There were Significant differences (p<.05) in soil pH, organic matter, total nitrogen, potassium, magnesium, available phosphorus and base saturation of the polluted soil and that of the control while the values of sodium, calcium, exchangeable acidity and electrical conductivity of the polluted soil did not show significant difference with that of the control. There was rather a steady increase which was not statistically different from the control with increase in concentration of the pollutant.The results of this study revealed that soil polluted with little quantities of palm oil mill effluent could enhance microbial proliferation and thus, increase soil fertility, while a heavy application inhibits the same.

Keywords: Effect, Effluent, Palm oil, Microflora, Fertility

Comment [DCO1]: Put it this way "In the present study, an agricultural soil artificially ammended with different concentrations of palm oil mill effluent was analysed to determine its impact on the soil microflora and other properties such as pH, organic matter, electrical conductivity, and mineral elements"

Comment [DCO2]: I expect the rest of the abstract and even the manuscript to be re-written this way!! The details about location of study and pattern of pollution should best be taken to the relevant sections of the materials and methods!!

Introduction

The world has entered a period of unprecedented environmental change as evidenced in the rapid growth of natural and man-made changes in the biosphere in recent times. Environmental Pollution causing changes in environmental quality isjust one issue raising widespread alarm. An increase in socio-economic activities worldwide has been accompanied by an even faster growth in pollution stress. This is because the majority of human needs can only be met with goods and services provided by industries. Industries have the capacity to improve as well as degrade the environment. Raw materials, which have been taken from the natural resource base, are converted into products by the industry. Lang [16]reported that during the production process pollutants are often released into the environment.

Comment [DCO3]: What exactly do you mean??

Soil is an efficient purifying medium with a great capacity to receive and decompose wastes matter by its microflora and precipitate out nutrients [23]. However, if the input of the pollutants exceeds the soil purifying limit, the effectiveness of soil microorganism activity is reduced considerably. As a result, there will be an adverse change in the soil physicochemical properties which consequently affect the growth and development of the crop plants.

Comment [DCO4]: Precipitate out??? No!! Nutrients are products of microbial decomposition of organic wastes but they are not precipitated out!!

Comment [DCO5]: 'Microbial' instead!!

Industrial effluents as pollutants contain a large number of both known and unknown substances formed during the production process. There is a direct impact of pollutants on minerals, organic matter and microbial community of soil [23]. The discharge of industrial effluents especially without treatment may have profound influence on physicochemical and biological properties of soil related to soil fertility.The palm oil industry is a major agro based industry in Nigeria especially in the Southern part where palm oil trees are found both in the

wild and plantations. During processing of the ripe fruits to extract the oil, a lot of wastewater (Palm Oil Mill Effluents – POME) is generated. It is estimated that for 1 tonne of crude palm oil produced, 5 - 7.5 tonnes of water ends up as POME [3]. The solid waste products that result from the milling operation are empty fruit bunches, palm fibre, and palm kernel. Much of the POME results from water used in processing [30][1]. Observations show that most of the POME is not treated before being discharged into the surrounding environment especially by the small scale mills, causing pollution problems.

POME, if untreated, contains high amounts of fatty acids, proteins, carbohydrates and other plant materials [27][6][11]. It alters the environmental parameters causing a change in water and soil quality especially in Biological Oxygen Demand, Dissolved Oxygen, Carbon/Nitrogen ratio and Chemical Oxygen Demand [30]. This has been supported by many researchers[21][2]. These parameters affect soil microbial flora which in turn affects soil fertility [5]. Soil fertility is a very important factor, considering the type of use a soil can be put into agriculturally. The raw or partially treated POME has an extremely high content of degradable organic matter, which is due in part to the presence of unrecovered palm oil [3]. This highly polluting wastewater can, therefore, cause pollution of waterways due to oxygen depletion and other related effects as reported bysome researchers[3]. Thus, while enjoying a most profitable commodity, palm oil, the adverse environmental impact from the palm oil industry cannot be ignored. It has been observed that most of the POME produced by the small-scale traditional operators undergoes little or no treatment and is usually discharged into the surrounding environment.

In recent years, economic boom in fast developing countries has been witnessed with spectacular progress in industrialization which has resulted in an increased generation of industrial wastes such as liquid, solid and volatile organic gases [3]. In an attempt to dispose of these, man has carelessly polluted the environment. These reasons have made it justifiable to assess the effects of POME on the microbial populations of the soil which enhances soil fertility.

The general objective of this study was to assess the effects of POME on microbial population and physicochemical properties of an agricultural soil.

Materials and methods

Experimental design

The soil used in this study was an agricultural soil in the University of Calabar. The pollution of the soil was performed artificiallywith four concentrations (0ml, 250ml, 500ml, 1000ml and 2000ml) of palm oil mill effluentin a completely randomized manner. And the duration of this study was sixteen weeks.

Collection of samples

Soil sample

Soil samples of 0-15cm depth were collected (5 kg)from five locations on an agricultural soil within university of Calabar by excavation using spade. The collected samples from all locations were thoroughly mixed on the spot in order to obtain composite sample. 5kg of this soil was weighed into four different polythene bags[29].

Palm Oil Mill Effluents Samples

POMEwas collected from Obasenjo's palm oil mill factory Mfamosing-Akamkpa, in Cross River State. The sample was collected with clean plastic containers rinsed several times with

Comment [DCO6]: Change to: "a lot of waste water called palm oil mill effluent (POME) is generated"

Comment [DCO7]: "Reports indicate" rather

Comment [DCO8]: Recaste!!

Comment [DCO9]: Taken from where in the University of Calabar??? There must be a location for your sampling!!

Comment [DCO10]: I do not understand why the authors chose 0mlof POME as control. In my opinion, POME should have been replaced with distilled water. Soil requires at least 50% moisture content in order to keep its microflora active and free from stress, dehydration and death. For an experiment that lasted 16 weeks, this is another major flaw!!! Another is that although different Conc of POME was used, the same volume of liquid would have been applied to maintain the soil moisture content in the respective treatments at the same value!!

Comment [DCO11]: Check the spelling!!

the same sample and transported within 24h to the Department of Soil Science, University of Calabar for analyses and pollution.

Pollution of the Soil Sample

Pollution of the soil was achieved by employing standard methods[31]. 0ml, 250ml, 500ml, 1000ml and 2000ml of theeffluent was added to 2 kg of already dried soil in each polythyene and mixed thoroughly for even distribution. These polluted soil and the unpolluted soil (control) were left outside under normal environmental condition for the duration of 16weeks (Four months)

Microbial analysis

Microbial analysis was carried out before and after pollution. 10g of Soil sample was collected aseptically, labelled and store in ice packed plastic coolers and transported to the Microbiology Department Laboratory University of Calabar where microbial analysis was carried out within 24h of sampling so as to maintain the stability of the sample without significant alteration in the microbial population[31].

Dilution

Serial dilution was carried out by weighing 10g of soil in to 90ml of sterile water contained in a stoppered 200ml volumetric flask and agitated to dislodge the microorganisms from the soil particles. From this initial dilution, a ten-fold serial dilution was prepared.

Enumeration of heterotrophic Bacteria

The counts of total heterotrophic bacteria in the soil samples were determined by pour plating 1ml of 10^{-5} dilutions into nutrient agar (NA). The medium was incorporated with antifungal agent (50µg/ml Nystatin), in order to prevent the growth of fungal contaminants. Bacterial colonies were counted after 24 h of incubation at room temperature and reported as a number of colony forming units (cfu/g) of soil[4].

Enumeration of heterotrophic Fungi

The total heterotrophic fungi count was measured by pour plating 1ml of 10^{-3}dilution into Sabouroud dextrose agar (SDA) supplemented with antibacterial agents (50µg/ml of streptomycin and 30µg/ml of penicillin) to inhibit the growth of bacterial contaminants. Fugal counts were reported after 72 h of incubation[4].

Enumeration of heterotrophic Actinomycetes

Enumeration of total actinomycetes was achieved by pour plate technique. 1ml of 10^{-2}dilution was plated unto sodium caseinate agar, 50µg/ml of nystatin and 30µm/ml of tetracycline was added to inhibit fungal and bacterial growth. An actinomycetes count was reported 7 days after incubation at room temperature[22][30].

Maintenance of pure isolates

Bacterial colonies were repeatedly transferred to freshly prepared nutrient agar plates by the streak-plate method and allow growing for 48 h before stocking. Similarly, distinct fungal and actinomycetes colonies were subculture repeatedly on freshly prepared Sabouroud dextrose agar plates and sodium caseinate agar, respectively. Pure isolates of the microorganisms were maintained on agar slants as stock, which were preserved in the refrigerator for further use.

Characterization and Identification of isolates

Comment [DCO12]: I prefer the term 'treatment' to your 'pollution'

Comment [DCO13]: Was the effluent treated before the treatment?? For instance, was it sieved to remove the solid fractions??? Soil was dried before the treatment, what about radiation, temperature changes and photochemical effect on microbial populations!! For 16 weeks there was no input of carbon for energy generation and mineral cycling. This a major flaw of this work!!

Comment [DCO14]: Describe correctly what you mean?? If the conditions of the experiment are not controlled, monitored or optimized, what about the influence of the elements on your results???

Comment [DCO15]: soil

Comment [DCO16]: stored

Comment [DCO17]: Is this distilled water, saline water or tap water. This detail need be provided!!

Comment [DCO18]: Delete!!

Comment [DCO19]: Check the spelling!!!

Comment [DCO20]: This is an ecosystem study. There was really no reason to identify the isolated microbes but if you must, then adopt molecular methods and then show the phylogenetic relationship of the isolated strains. The method reported here is unacceptable and merely speculative!!!!

Various methods were used to characterize and identify the isolates[4][15][40]. The test results for bacteria were evaluated using characteristics presented in *Bergy's Manual of Determinative Bacteriology* [15].

Comment [DCO21]: What are these references for or about???

Representative colonies of fungal isolates were characterized and identified based on their cultural and morphological features. The characterizations were achieved through staining techniques-using lactophenol in cotton blue[4][15][40].

Soil Physicochemical analysis

Physicochemical analysis of the pristine soil was carried out before and after pollution with varying concentrations of the oil mill effluent, physiochemical analysis of each soil sample polluted with different concentrations of each effluent was also carried out bimonthly (every 8weeks). Physicochemical analysis was assessed in terms of the following parameters:

Particle size and textural class analysis

In carrying out this test, the Bouyoucos-type hydrometer method was used [12].

Soil pH

Soil pH was determined in water 1:2 soils: water ratio using pH meter with glass electrode. 20g of air-dried soil was weighed into a 50ml beaker, and 20ml of sterile saline water was added and allowed it to stand for 30mins. The electrode of the pH meter was inserted into the 1:2soil /water partly settled suspension and measured the pH. The result was recorded as soil pH measure in water[40].

Electrical conductivity (EC)

In the same soil solution (1:2 soil /water solution) for pH determination, electrical conductivity electrode was inserted into the partly settled suspension and the EC was measured[40]

Organic matter

This wasdetermined by the dichromate wet-oxidation method [26].

Total nitrogen

Total nitrogen was determined by the micro- Kjeldahl method [9].

Available phosphorus

Available phosphorus was extracted with acid fluoride using Bray P-1 method [8]

Exchangeable cations

The bases were extracted with neutral NH_4OAC. Calcium and magnesium were determined in the extract by EDTA titration, and potassium and sodium by the use of flame photometer [39].

Exchangeable acidity

A+ and H+ were obtained by leaching the soil with INKCl solution and the and the extract titrated with standard NaOH as described by I.I.T.A (1979).

Exchangeable acidity (Al + H) – Exchangeable Al = Exchangeable H

Effective cation exchange capacity

This was determined by calculation. That is, total exchangeable bases (Ca+ Mg + Na) + Exchangeable acidity (EA).

Percentage base saturation

This will be achieved by dividing the total exchangeable bases by exchangeable cation capacity and multiplied by 100 [6].

$$\% \text{ base saturation} = \frac{\text{summation of exchangeable bases} \times 100}{\text{ECEC}}$$

Statistical analysis

All statistical analysis of data from various treatments was carried out using analysis of variance (ANOVA) test using factorial experiment. Means were separated using least significance difference (LSD).

Results

Microbial analyses

Table 1 below shows the enumeration of total heterotrophic bacteria (THB), total heterotrophic fungi (THF) and total heterotrophic actinomycetes (THA) in the pristine soil. The counts obtained were as follows $1.90 \pm 1.41 \times 10^7$ Cfu/g, $1.29 \pm 1.25 \times 10^5$ cfu/g and $9.2 + 1.25 \times 10^3$ cfu/g respectively.

TABLE 1

Total microbial counts of the soil before the commencement of the study

THB (cfu/g)	$1.90 \pm 1.41\times10^7$
THF (cfu/g)	$1.29 \pm 1.25\times10^5$
THA (cfu/g)	$9.2 \pm 1.25\times10^3$

Key:*THB = Total heterotrophic bacteria, THF= Total heterotrophic fungi, THA= Total heterotrophic actinomycetes, cfu/g = Colony forming unit/gram*

Table 2 below shows the effect of different concentrations of palm oil mill effluent on microbial population of POME- polluted soil.The results obtained for THB in 250ml, 500ml, 1000ml and 2000ml were $1.59 \pm 1.84 \times 10^7$ cfu/g, $1.24 \pm 1.40 \times 10^7$cfu/g, $7.0 \pm 1.38 \times 10^6$ cfu/g and $5.9 \pm 1.22 \times 10^6$ cfu/g respectively. The results showed that there was no significant difference ($p<.05$) in the mean counts of THB obtained in 250ml and 500ml, and no significant difference ($p<.05$) was also observed in THB counts of 1000ml 2000ml of the polluted soil (Table 2).

The results for the counts of THF in different concentrations showed that there was a significant increase ($p < 0.05$) in total heterotrophic fungi (THF) counts of 250ml and 500ml with mean counts of $2.02 \pm 3.00 \times 10^5$cfu/g, $1.71 \pm 1.89 \times 10^5$ cfu/g respectively over the control, and a significance reduction ($p>.05$) in THF counts of 1000ml and 2000ml with mean counts of $1.07 \pm 1.26 \times 10^5$ cfu/g and $5.1 \pm 1.42 \times 10^4$ cfu/g respectively (Table 2).

The total heterotrophic actinomycetes (THA) counts in different concentrations showed that, there wasa significant difference ($P <.05$) in THA counts among the concentrations. There was a significant increase ($p<.05$) in THA counts in 250ml and 500ml with mean counts of $1.33 \pm 2.09 \times 10^4$ cfu/g, $1.41 \pm 1.84 \times 10^4$ cfu/g respectively over the control with mean count of $8.9^b \pm 1.73 \times 10^3$cfu/g while there was no significance($p>.05$) in THA in 1000ml and 2000ml with mean counts of $4.8 \pm 1.16 \times 10^3$ cfu/g and $3.6^b \pm 1.09 \times 10^3$ cfu/g respectively over the control (Table 2).

TABLE 2
Effect of concentrations of pollution on microbial population in palm oil mill polluted soils

	0ml	250lm	500ml	1000ml	2000ml
THB (cfug^{-1})	$1.89^{a}\pm2.90x10^{7}$	$1.59^{a}\pm1.84x10^{7}$	$1.24^{a}\pm1.40x10^{7}$	$7.0^{b}\pm1.38x10^{6}$	$5.9^{b}\pm1.22x10^{6}$
THF (cfug^{-1})	$1.26^{c}\pm2.51x10^{5}$	$2.02^{b}\pm3.00x10^{5}$	$1.71^{b}\pm1.26x10^{5}$	$1.07^{c}\pm1.26x10^{5}$	$5.1^{d}\pm1.42x10^{4}$
THA (cfug^{-1})	$8.9^{b}\pm1.73x10^{3}$	$1.33^{a}\pm2.09x10^{4}$	$1.14^{a}\pm1.84x10^{4}$	$4.8^{b}\pm1.16x10^{3}$	$3.6^{b}\pm1.09x10^{3}$

Key: means with the same letter along the horizontal arrays indicates significant difference (P< 0.05), and means with different letter along the column indicates no significance difference (P>0.05)
THB = Total heterotrophic bacteria, THF= Total heterotrophic fungi, THA= Total heterotrophic actinomycetes, cfu/g = Colony forming unit/gram

Table 3 below shows the effect of POME and duration of pollution on microbial populations. The total heterotrophic bacteria (THB) counts after 8 weeks and 16 weeks of pollution were $1.62 \pm 4.21 \times 10^{7}$ cfu/g and $7.84 \pm 0.98 \times 10^{6}$ cfu/g respectively. Significant reduction (p>.05) was observed in 16 weeks of pollution (Table 3). Also the counts obtained for total heterotrophic fungi (THF) after 8 weeks of pollution produced a mean count of $1.56 \pm 3.81 \times 10^{5}$ cfu/g was significantly higher than the THF counts after 16 weeks of pollution with a mean count of $1.07 \pm 2.00 \times 10^{5}$cfu/g(Table 3). And for the effect of duration of pollution on total heterotrophic actinomycetes (THA) counts obtained, there was no significant difference (P>.05) in the count obtained after 8 weeks and 16 weeks of pollution.

TABLE 3
Effect of duration of pollution on microbial population

	8 weeks	16 weeks	LSD
THB(cfu/g^{-1})	$1.62 \pm 4.21 \times 10^{7\,a}$	$7.84 \pm 0.98 \times 10^{6\,b}$	2.69
THF (cfu/g^{-1})	$1.56\pm 3.81 \times 10^{5\,a}$	$1.07\pm 2.06 \times 10^{5\,b}$	3.38
THA (cfu/ g^{-1})	$9.4 \pm 1.28 \times 10^{3\,b}$	$7.4\pm 1.11 \times 10^{3\,b}$	4.04

Key: means with the same letter along the horizontal arrays indicates significant difference (P< 0.05), and means with different letter along the column indicates no significance difference (P>0.05)
THB = Total heterotrophic bacteria, THF= Total heterotrophic fungi, THA= Total heterotrophic actinomycetes, CFU/g = Colony forming unit/gram, LSD= Least significant difference

Characterization and Identification of Microbial Isolates

Microbial isolates were characterized and identified based on their morphological and biochemical characteristics. The following bacteria were isolated from POME polluted soil; *Pseudomonas spp., Serratia spp., Bacillus spp., Micrococcus spp., Proteus spp., while the fungal isolates were Rhizopus spp., Aspergillus spp., Mucor spp., Penicillium spp.*

Soil pH

The pH values of the control (0ml) soils were in the range of 5.66 ± 0.50 to 5.7 ± 0.21. The pH values in POME treated soils ranged from 6.28 ± 0.19 to 6.83 ± 0.24 There was no significant difference (p <.05) in the pH value of the soils treated with 250ml, 500ml and 1000ml POME and in the soil treated with 250ml, 500ml, 1000ml and 2000ml, but there was a significant difference (p>.05) in the pH value of the soil treated with 2000ml of the effluent. There was significant difference (p > 0.05) in the pH values over the duration of pollution (Figure 1).

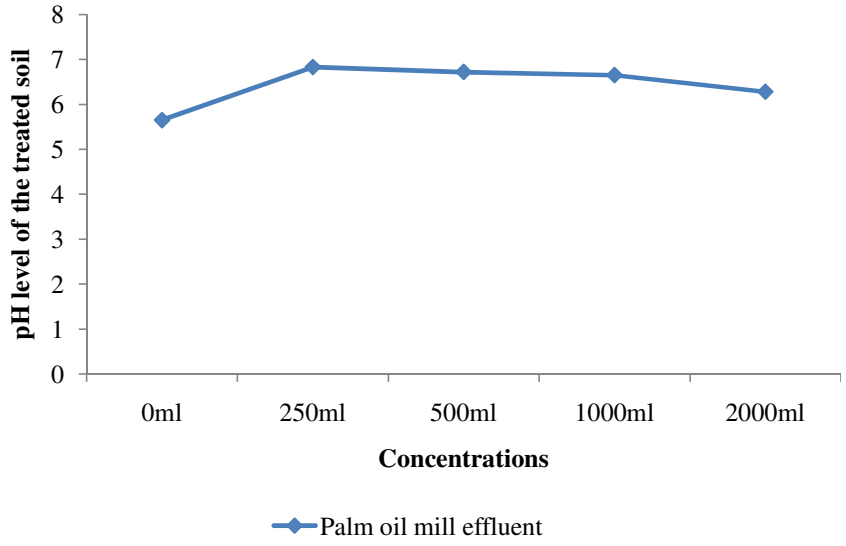

Figure 1: Effects of concentrations of palm oil mill effluent on the soil pH

Organic Matter Content

The values of organic matter in control (0ml) soils were in the range of 2.41 ± 0.23% and 2.41 ± 0.09%. The organic matter content of the soils treated with varying concentrations of POME ranged from 3.53 ± 0.19% to 4.97 ± 0.26% (Table 6 and Figure 2).

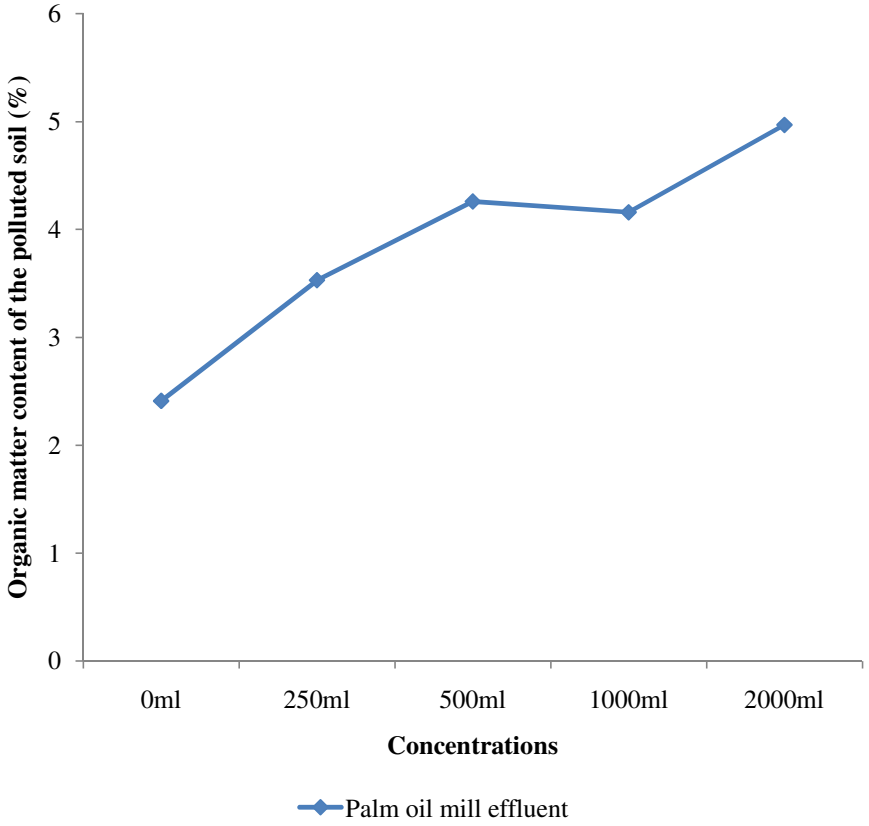

Figure 2: Effects of concentrations of palm oil mill effluents on soil organic matter

Total Nitrogen Content

The control soils (0ml) had the total nitrogen content in the range of 0.06 ± 0.2% and 0.06 ± 0.01%. In the soil treated with varying concentrations of POME, the total nitrogen content ranged from 0.08 ± 0.01% to 0.10 ± 0.04%. Significant difference ($p<.05$) was not observed among concentration There was no significant difference in total nitrogen content over the duration of study,

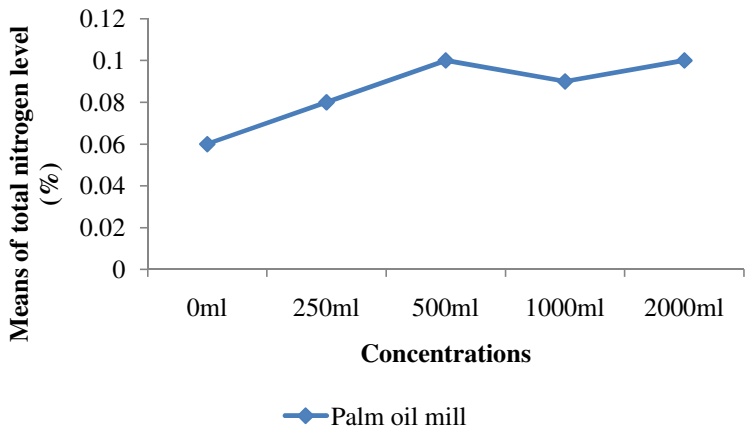

Figure 3: Effects of concentrations of palm oil mill effluent on total nitrogen content of the polluted soil

Effective Cation Exchange Capacity (ECEC)

The values of ECEC in the control (0ml) soils were in the range of 9.45 ± 0.43 cmol/kg and 9.7 ± 0.06 cmol/kg (Table 5). The value of ECEC in palm oil mill effluent polluted soil increased to between 9.8 ± 0.14cmol/kg and 11.55 ± 0.30 cmol/kg (Table 6 and Figure 3). There was significant increase ($p < .05$) in ECEC in 2000ml polluted soil and significance difference ($p > .05$) did not exist in 250ml ,500ml, 1000ml and that of 250ml, 500ml 1000ml.

TABLE 4

Physicochemical properties of the soil before the commencement

Parameters	Values
PH	5.60
Electrical conductivity (ds/m)	0.055
Organic matter (%)	2.41
Total nitrogen (%)	0.06
Available Phosphorus (mg/kg)	31.54
THC (mg/kg)	BDL
Ca (cmol/kg)	4.00
Mg (cmol/kg)	2.10
Na (cmol/kg)	0.06
K (cmol/kg)	0.21
EC (cmol/kg)	1.11
ECEC (cmol/kg)	8.82
Base saturation (%)	85.16
Sand (%)	75.80

Comment [DCO22]: What about the soil moisture content??

Comment [DCO23]: What is this??? Potency of hydrogen or Port-harcout!!!

Silt (%)	12.60
Clay (%)	11.60
Lead (mg/kg)	6.20
Nickel (mg/kg)	9.44
Cadmium (mg/kg)	4.32
Chromium (mg/kg)	5.60
Iron (mg/kg)	919.66

TABLE5

Effects of concentrations of pollution on the physiochemical properties of the soil polluted with palm oil mill effluent

	0ml	250ml	500ml	1000ml	2000ml	LSD
pH	$5.65^{c}\pm0.50$	$6.83^{a}\pm0.24$	$6.72^{a}\pm0.20$	$6.65^{a}\pm0.18$	$6.28^{b}\pm0.19$	0.20
Organic matter	$2.41^{e}\pm0.23$	$3.53^{c}\pm0.19$	$4.26^{b}\pm0.26$	$4.16^{b}\pm0.26$	$4.97^{a}\pm0.26$	0.18
Avail. P	$31.54^{a}\pm2.64$	$71.1^{c}\pm1.24$	$14.49^{b}\pm2.02$	$11.49^{f}\pm1.05$	$15.42^{d}\pm1.18$	0.85
Total N	$0.06^{c}\pm0.02$	$0.08^{b}\pm0.01$	$0.10^{b}\pm0.03$	$0.09^{b}\pm0.02$	$0.10^{b}\pm0.04$	0.02
Mg	$2.37^{d}\pm0.09$	$2.73^{b}\pm0.11$	$2.13^{e}\pm0.08$	$2.64^{c}\pm0.14$	$3.25^{a}\pm0.09$	0.09
Sodium (Na)	$0.09^{b}\pm0.03$	$0.11^{b}\pm0.01$	$0.11^{b}\pm0.02$	$0.12^{b}\pm0.01$	$0.12^{b}\pm0.04$	0.04
Calcium (Ca)	$4.75^{a}\pm0.10$	$5.18^{a}\pm0.9$	$5.31^{a}\pm0.08$	$5.40^{a}\pm0.05$	$5.50^{a}\pm0.08$	0.61
Ec	$0.05^{b}\pm0.01$	$0.13^{a}\pm0.02$	$0.14^{a}\pm0.01$	$0.15^{a}\pm0.03$	$0.17^{a}\pm0.02$	0.02
Base saturation	$78.52^{b}\pm2.01$	$78.32^{b}\pm1.53$	$78.06^{b}\pm0.69$	$78.27^{b}\pm0.89$	$80.09^{a}\pm1.14$	1.35
K	$0.21^{b}\pm0.02$	$0.29^{a}\pm0.03$	$0.23^{b}\pm0.01$	$0.16^{b}\pm0.09$	$0.29^{a}\pm0.08$	0.02
EFEC	$9.45^{b}\pm0.43$	$10.61^{b}\pm0.21$	$9.8^{b}\pm0.14$	$10.63^{b}\pm0.08$	$11.55^{a}\pm0.30$	0.69
EA	$2.03^{a}\pm0.8$	$2.3^{a}\pm0.05$	$2.15^{a}\pm0.04$	$2.31^{a}\pm0.08$	$2.3^{a}\pm0.02$	0.20

Key: means with the same letter along the horizontal arrays indicates significant difference (P< 0.05), and means with different letter along the column indicates no significance difference (P>0.05)

358
359
360
361 **TABLE 6**

362 **Effects of duration of pollution on physico- chemical properties of the soil polluted with palm oil effluent**

	8 weeks	16 weeks	LSD
pH	$6.6^{b}\pm0.08$	$6.25^{d}\pm0.04$	0.13
Organic matter	$4.02^{a}\pm0.10$	$3.71^{b}\pm0.09$	0.11
Avail. P	$18.31^{b}\pm0.24$	$19.7^{a}\pm0.36$	0.54
Total N	$0.09^{b}\pm0.02$	$0.08^{b}\pm0.02$	0.01
Mg	$2.87^{a}\pm0.10$	$2.37^{c}\pm0.05$	0.06
Na	$0.13^{b}\pm0.02$	$0.09^{c}\pm0.02$	0.02
Ca	$5.38^{a}\pm0.11$	$5.00^{a}\pm0.09$	0.38
Ec	$0.13^{a}\pm0.02$	$0.13^{a}\pm0.01$	0.01
Bs	$79.19^{a}\pm2.10$	$78.25^{a}\pm1.80$	0.86
K	$0.22^{b}\pm0.02$	$0.25^{a}\pm0.03$	0.01
ECEC	$10.86^{a}\pm0.28$	$10.02^{b}\pm0.20$	0.44
EA	$2.26^{a}\pm0.09$	$2.18^{a}\pm0.08$	0.13

363
364 *Key: means with the same letter along the horizontal arrays indicates significant difference (P< 0.05), and means with different letter along the*
365 *column indicates no significance difference (P>0.05).*

Discussion

Soil is a potent system of terrestrial ecosystem and direct discharge of industrial effluents especially that without treatment may have profound influence on physicochemical and biological properties of soil related to soil fertility. The pollutant level of POME varies with the quality of the raw material and production process used to manufacture the palm oil.

Comment [DCO24]: This is not clear. Pls break into two separate sentences!!

In this study, following pollution of soil with varying concentrations of POME, the soil showed profound changes in the microbial populations and physicochemical properties.In assessing the effects of different concentrations of POME on microbial population, the result showed no significant difference ($p > .05$) in THB mean count in 250ml and 500ml of effluent polluted soil over the controls. This implies that the THB mean counts in 250ml and 500ml the polluted soil fall in the same range with that of the control. Also, there was a significant reduction ($p \leq .05$) in THB in 1000ml and 2000ml over the control. These indifferences in THB mean count possesses some similar characteristics and hence have the same effect on soil bacteria. Also, the result showed that there was a significant increase ($p \geq .0\ 5$) in THF and THA in 250ml and 500ml (Table 2). This increase in fungi population may be attributed to the acidity nature of the effluents, because fungi strive best in acidic environments than bacteria which can only survive in a neutral to alkaline pH environment. Significant increase ($p \geq .05$) in THA count was also observed in 250ml and 500ml which had THA count higher than the controls. This may also be attributed to the acidic nature of the effluents and the soil since soil actinomycetes have been known to tolerate acidic environment than the true bacteria. Soil actinomycetes can tolerate a pH level of up to 6.80, and this was the pH level in most of the treated soils in this study. An increase in microbial population has earlier been reported [30].

In terms of concentrations of pollution, 250ml of the effluents was found to be most favourable concentration for the proliferation of microorganism, followed by 500ml in this study. Similar result had earlier been reported [29].It was observed that effluent impacted soil at low concentration encouraged proliferation of microorganisms but at high concentration, inhibits the same [29],this wasalso observed in this study (Table 2). The components of the POME used in this study revealed that the effluents contain some metabolizable nutrients for the soil microorganisms and excess of these nutrient with the high water content suppressed the microorganisms. Excess water in soil restricts microorganisms and their activities by preventing oxygen movement into and through the soil in sufficient quantity to meet the oxygen demand of the microorganisms [33]. Biodegradation of pollutants in the soil requires oxygen but such oxygen was not available in 1000ml and 2000ml of POME polluted soil used in this study.

Comment [DCO25]: What were the reasons for this observation!!

Comment [DCO26]: Present appropriate references to justify this claim!!

Comment [DCO27]: This is not acceptable. This can only occur under extremely anerobic condition!!

Also in assessing the effect of duration of pollution and different concentrations of pollution on microbial population, THB showed significant increase ($p > 0.01$) with decrease in concentrations of pollutions and significant reduction with increase in the duration of pollution (Table 3). This result implies that there was a steady reduction in THB with increase in concentrations and duration of pollution. Also, the THF mean counts at 250ml showed significant increase ($p > .05$) after 8 weeks of pollution and a significant reduction was observed in the same concentration of pollution after 16 weeks of pollution and while there was no significant difference in THF count at 500ml, 1000ml of after 8 weeks and 16 weeks of pollution (Table 3). Similarly, there was significant increase ($p > .05$) in THA at 250ml, 500ml, after 8 weeks of pollution and 250ml after 16weeks of pollution, and no significant difference was also observed in THA in 1000ml, 2000ml after 8 weeks of pollution and 500ml, 1000ml and 2000ml after 16weeks of pollution. This result implies that,

there was a significant increase ($p > .05$) in THB, THF and THA in all the soilsamples, followed by a reduction in THB and THF after 16 weeks of pollution except THA which did not show reduction after 16 weeks of pollution (Table 3).

The result of this study also showed changes in physicochemical properties. This change has earlier been reported that continual applications of effluents on the soil can change soil properties, e.g. pH and nutrient concentrations [37].
The result also showed that there was a significant increase ($p > .05$) in the pH values in all the soil polluted with POME over the control. Increase in pH in POME polluted soil is in agreement with the report of Okwute and Isu[30], who reported that when raw POME is discharged into the soil, the acidic pHseems to gradually increase to alkaline as biodegradation takes place, and this was the case in this study as pH of the polluted soils increases from 5.65 ± 0.05 in the control to between 6.28 ± 0.19 and 6.83 ± 0.24. The increase in pH of POME polluted soil has also been earlier reported [29][18].

The polluted soils were also characterised by increased in organic matter and total nitrogen. Organic matter of a soil is usually determined and reported as a measure of organic carbon. Organic matter content strongly affects soil fertility by defining the energy supply to microorganisms, availability and quality of substrates and the biodiversity necessary to sustain much soil functions[26][40][41]

Conclusion

This study on the effects of palm oil mill effluent on soil microflora and fertility showed that the mean counts of total heterotrophic bacteria, total heterotrophic fungi and total heterotrophic actinomycetes increased with decrease in concentration of pollution and decreased with increase in the duration of pollution in the soils.Similarly, the POME also caused profound changes on the soil physicochemical properties of the polluted soils.
The result revealed significant differences in soil pH, organic matter content, total nitrogen, available phosphorus, magnesium, electrical conductivity, percentage base saturation, potassium and effective cation exchange capacity between polluted and non-polluted soil while no significant difference was observed in calcium, sodium and exchangeable acidity between polluted and non-polluted soil.

In conclusion, this study revealed that POME could be harmful to the soil if not properly managed and also useful to the soil if properly discharged since light application of the effluents could enhance microbial proliferation which enhances soil fertility. Therefore, government should create awareness to those involved in small and large scale palm oil processing on the need for proper disposal of effluent because if not properly managed, it can negatively affect soil fertility by inhibiting microbial proliferation.

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