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# Evaluation of the Microbial Status of Palm Oil Mill Effluents Contaminated Soil in Ebonyi State, Nigeria

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**Abstract:** Palm oil mill effluent (POME) is one of the chief wastes generated from palm oil mills and can constitute environmental pollution if not properly disposed or treated before disposal. POME contaminated soil, non-POME contaminated soil and raw POME from Abakaliki, Ebonyi, Ikwo, Ishielu and Ohaukwu LGAs were evaluated for microbial and physiochemical analysis using standard procedures. The highest bacterial loads were obtained from non contaminated soil (6.4 $X10^6$ cfu/g, 7.0 $X10^6$ cfu/g, 7.0 $X10^6$ cfu/g, 6.9 $X10^6$ cfu/g and 6.6. $X10^6$ cfu/g), followed by POME contaminated soil (5.6 $X10^5$ cfu/g, 5.8 $X10^5$ cfu/g, 5.7 $X10^5$ cfu/g and 5.5 $X10^5$ cfu/g, 5.7 $X10^5$ cfu/g), while raw POME recorded the least (2.8 $X10^4$ cfu/g, 2.5 $X10^4$ cfu/g, 2.7 $X10^4$ cfu/g, 2.8 $X10^4$ cfu/g and 2.9 $X10^4$ cfu/g) for Abakaliki, Ebonyi, Ikwo, Ishielu and Ohaukwu LGAs respectively. 155 bacterial (22 *Pseudomonas* spp., 20 *Bacillus* spp., 19 *Flavobacterium* spp., 16 *Arthrobacter* spp., 15 *Micrococcus* spp., 13 *Corynebacterium* spp., 11 *Staphylococcus* spp., 9 *Proteus* spp., 9 *Klebsiella* spp., 8 *Citrobacter* spp., 7 *Streptococcus* spp., 6 *Enterobacter* spp., isolates were obtained from non-POME contaminated soil, 95 (18 *Pseudomonas* spp., 15 *Bacillus* spp., 13 *Arthrobacter* spp., 11 *Flavobacterium* spp., 11 *Micrococcus* spp., 10 *Corynebacterium* spp., 6 *Citrobacter* spp., 5 *Enterobacter* spp., 5 *Staphylococcus* spp.) from POME contaminated soil and 44 (14 *Bacillus* spp., 10 *Pseudomonas* spp., 10 *Micrococcus* spp. and 10 *Flavobacterium* spp.) from raw POME. Five fungal isolates (*Aspergillus* spp., *Candida* spp., *Penicillium* spp., *Mucor* spp. and *Fusarium* spp.) were obtained from this work were identified. There was increase in conductivity, oil and grease, exchangeable cations (Na, K, $Ca^{2+}$ and $Mg^{2+}$), total organic carbon, phosphates and nitrates in POME contaminated soils when compared with non-POME contaminated soil samples and the raw POME. Higher sulphate and pH were recorded in non-POME contaminated soil than POME contaminated soil and raw POME. There was increase in hydrocarbon utilizing microbial loads from the POME contaminated soil, non-POME contaminated soil and the raw POME. POME could have a positive effect due to its increase in most physicochemical parameters which in return increases soil fertility if discharged properly.

**Key words:** Microbial species · Palm Oil Mill Effluents (POME) · Contaminated Sites · Non Contaminated Sites

## INTRODUCTION

Soil is the key component of natural ecosystem [1] and as such environmental sustainability depends largely on a sustainable soil ecosystem [2]. Due to soil chemical composition and physical properties soil forms a habitat for massive amounts of microorganisms and other living organisms [3].

Oil palm (*Elaeis guineensis*) is one of the species of palm oil commonly called African oil palm or "macaw fat" [4]. It is the primary source of palm oil [5]. Oil palm is the oil that is contained in oil palm fruit pulp. The fruit attached to a rachis or an empty bunch, the assembly forming the big cluster or bunch. These bunches are the raw materials used in palm oil mill. They are harvested from oil palms in equatorial regions. Bunches are raw materials of agriculture origin with variable characteristics. The fruit is fragile and perishable so its quality depends to a great extent on the conditions of growth, harvest and transport to the factory, which are the responsibilities of the mill operator [6]. The global production of palm oil is growing at a very high rate and the pollution caused by waste materials from the palm oil mills has become a serious problem [7]. Nevertheless, wet process of palm oil

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milling consumes a huge quantity of process water. It is estimated that about 5-7.5 tones of water is required for the production of 1 tone of crude palm oil but more than 50% of the water will end up as palm oil mill effluent (POME) [8].

POME is the voluminous liquid waste that comes from the sterilization and clarification sections of the oil palm milling process [9]. POME is one of the major wastes from the palm oil industry and it has the most problematic environmental pollution potential among the palm oil mill wastes [10]. Observations show that most of the POME is not treated before discharged into the surrounding environment especially by the small-scale mills, causing pollution problems [11].

POME application to soil can result to some beneficial soil chemical and physical characteristics, such as increases in organic matter, organic carbon, major nutrients (e.g. N, P), water-holding capacity and porosity [12 - 14]. However, it brings about undesirable changes such as decreases in pH and increases in salinity etc [15]. These effects occur very slowly and need many years to provide significant results. Soil microbiological and biochemical properties have been considered early and sensitive indicators of soil changes and can be used to predict long-term trends in the quality of soil [16]. Soil microbial properties are equally affected by environmental factors [17], in the same vein [18] reported that high rate of inorganic fertilizer application suppresses microbial respiration and dehydrogenase activity. Other factors as increases in salinity or decreases in water availability may also reduce biological activity [19].

Much information is not known on the impact of POME on the microbial qualities of the soil. Hence this present work designed to evaluate the microbial status of soil contaminated POME in Ebonyi state.

## MATERIALS AND METHODS

**Sample Sites:** The sample sites are the palm oil mills located at Abakaliki, Ebonyi, Ikwo, Ishielu and Ohaukwu Local Government Areas (LGA) of Ebonyi State, Nigeria. Each of the oil mills discharge their palm oil mill effluent in open land space near the mill. The co-ordinates of Ebonyi state are Latitude 06° 4′N and longitude 08° 5′E [20].

**Sample Collection:** Thirty soil samples, six from each LGA were aseptically collected. The soil samples were collected from the POME dump sites with the aid of disinfected trowel from 0-15cm depths and non-contaminated soil samples collected 10 yards away from the effluent site. Soil samples were immediately transferred to the laboratory for their microbial and physicochemical content analysis.

**Microbiological Analysis of the Soil Samples:** Fungi and bacteria were isolated from the soil samples by the standard spread plate technique [21]. One gram of each of the composite soil samples was added to 9 ml of sterile normal saline in a flask and shaken very well. The suspension obtained was diluted to $10^{-7}$ by serial dilution. Using pour plate method as described by Ofomata [22], one milliliter aliquots of the dilutions were spread on the surface of plates of Sabouraud dextrose agar (SDA) which was added 25mg of chloramphenicol tablets incubated at 25°C for 72 hours for fungi growth and on nutrient agar (NA) at 27°C for 48 hours for bacteria growth by pour plate method as described by Ojonoma and Udeme [23]. Discrete colonies of aerobic bacteria and fungi were subculture for purification by streaking on fresh solid media (Nutrient agar) for bacteria while fungi colonies were subculture in sabouraud dextrose agar which chloramphenicol was added. The culture plates were incubated at 27°C for 48 hrs for bacteria and at 25°C for 72hrs for fungi.

**Physicochemical Analysis of Soil Samples:** A number of physicochemical parameters of the contaminated soil and non-contaminated samples were determined. They included pH, temperature, conductivity, nitrate, phosphate and sulphate. Others included oil and grease, total organic carbon and exchangeable cations. Hach pH meter (Model EC10) was used for pH measurement; conductivity was measured using Hach conductivity meter (Model CO150). Sulphates, nitrates and phosphates were determined using Barium chloride (Turbidimetric method), Cadmium reduction and Ascorbic acid methods respectively. All analyses were in accordance with American Public Health Association [1].

## RESULTS

Result of the bacteria load of the samples revealed that the non POME contaminated soil (control) has the highest bacterial load (Abakaliki, $6.4X10^6$ cfu/g; Ebonyi, $7.0X10^6$ cfu/g; Ikwo, $7.0 X10^6$ cfu/g; Ishiaelu, $6.9X10^6$ cfu/g and Ohaukwu, $6.6X10^6$ cfu/g), followed by POME contaminated soil (Abakaliki, $5.6X10^5$ cfu/g; Ebonyi, $5.8X10^5$ cfu/g; Ikwo, $5.7X10^5$ cfu/g, Ishielu, $5.5X10^5$ cfu/g

Table 1: Bacterial load of the samples (non-POME contaminated soil, POME contaminated soil and raw palm oil mill effluent)

| Samples | Sample Location: Abakaliki | Ebonyi | Ikwo | Ishielu | Ohaukwu |
|---|---|---|---|---|---|
| Non-contaminated soil ($X10^6$ cfu/g) | 6.4 | 7.0 | 7.0 | 6.9 | 6.6 |
| Contaminated soil ($X10^5$ cfu/g) | 5.6 | 5.8 | 5.7 | 5.5 | 5.7 |
| Raw POME ($X10^4$ cfu/g) | 2.8 | 2.5 | 2.7 | 2.8 | 2.9 |

Table 2: Fungal load of the samples (Non POME contaminated soil, POME contaminated soil and raw oil palm mill effluent)

| | Sample Location: Abakaliki | Ebonyi | Ikwo | Ishielu | Ohaukwu |
|---|---|---|---|---|---|
| Non-POME contaminated soil ($X10^3$ cfu/g) | 3.2 | 3.1 | 3.0 | 3.3 | 3.2 |
| POME Contaminated soil ($X10^3$ cfu/g) | 2.1 | 2.3 | 2.0 | 1.9 | 2.3 |
| Raw POME ($X10^3$ cfu/g) | 1.3 | 1.3 | 1.2 | 1.3 | 1.1 |

Table 3: Characteristics of the bacteria isolates from inorganic fertilizer enriched soil

| Cell Morphology | Gram Reaction | Motility | Catalase | Indole | Oxidase | Coagulase | Citrate | Suspected Organism |
|---|---|---|---|---|---|---|---|---|
| Rod shaped | + | - | + | - | + | - | + | *Bacillus* species |
| Rod shaped | - | - | + | - | + | - | + | *Pseudomonas* species |
| Cocci | + | - | + | - | - | + | - | *Staphylococcus* species |
| Rod shaped | - | + | + | + | - | - | - | *Proteus* species |
| Rod shaped | - | + | + | - | - | - | - | *Citrobacter* species |
| Cocci | - | + | + | - | - | - | + | *Streptococcus* species |
| Rod shaped | + | - | - | - | - | + | - | *Flavobacterium* species |
| Rod shaped | - | - | + | + | + | - | - | *Arthrobacter* species |
| Cocci | - | - | + | - | - | - | - | *Micrococcus* species |
| Rod shaped | + | - | + | - | + | + | - | *Corynebacterium* species |
| Rod shaped | + | - | + | + | - | - | + | *Enterobacter* species |
| Rod shaped | - | + | + | - | - | - | + | *Klebsiella* species |

Key: + = Positive, - = Negative

and Ohaukwu, 5.7X10$^5$ cfu/g), while the raw palm oil mill effluent (Abakaliki, 2.8X10$^4$ cfu/g; Ebonyi, 2.5X10$^4$ cfu/g Ikwo, 2.7X10$^4$ cfu/g, Ishielu, 2.8X10$^4$ cfu/g and Ohaukwu, 2.9X10$^4$ cfu/g) recorded the least in all the sample locations studied as shown in Table 1.

The fungal load of the samples revealed that the non POME contaminated soil (control) has the highest bacterial load (Abakaliki, 3.2X10$^3$ cfu/g; Ebonyi, 3.1X10$^3$ cfu/g; Ikwo, 3.0X10$^3$ cfu/g; Ishiaelu, 3.3X10$^3$ cfu/g and Ohaukwu, 3.2X10$^3$ cfu/g), followed by the POME contaminated soil (Abakaliki, 2.1X10$^3$ cfu/g; Ebonyi, 2.3X10$^3$ cfu/g; Ikwo, 2.0X10$^3$ cfu/g, Ishielu, 1.9X10$^3$ cfu/g and Ohaukwu, 2.3X10$^3$ cfu/g), while the raw palm oil mill effluent (Abakaliki, 1.3X10$^3$ cfu/g; Ebonyi, 1.3X10$^3$ cfu/g; Ikwo, 1.2X10$^3$ cfu/g, Ishielu, 1.3X10$^3$ cfu/g; Ohaukwu, 1.1X10$^3$ cfu/g) recorded the least in all the sample locations studied as shown in Table 2.

Result of the morphological and biochemical characteristics of the bacterial isolates showed that *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species, *Proteus* species, *Citrobacter* species, *Streptococcus* species, *Flavobacterium* species, *Arthrobacter* species, *Micrococcus* species, *Corynebacterium* species, *Enterobacter* species *and Klebsiella* species were isolated from the non contaminated (control) soil samples while *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species, *Citrobacter* species, *Flavobacterium* species, *Arthrobacter* species, *Micrococcus* species, *Corynebacterium* species and *Enterobacter* species were isolated from the soil contaminated with oil palm mill effluents. The organisms isolated from the raw oil palm mill effluents include species, *Pseudomonas* species, *Bacillus* species, *Flavobacterium* species and *Micrococcus* species (Table 3).

Table 4 showed the percentage distribution of the bacterial species isolated from the samples (oil palm mill effluent contaminated soil, non contaminated soil (control) and raw oil palm mill effluent). Out of the 155 bacterial isolates obtained from non-contaminated soil, *Pseudomonas* species recorded (14.2%) highest bacterial, followed by *Bacillus* species (12.90%), *Flavobacterium* species (12.3%), *Arthrobacter* species (10.3%) while *Enterobacter* species (3.90%) recorded the least.

Table 4: Percentage distribution of the bacterial species isolated from the oil palm mill effluent contaminated soil, non contaminated soil (control) and raw oil palm mill effluent

| Microorganisms Isolated | Non-contaminated soil (%) | Contaminated soil (%) | Raw POME (%) |
|---|---|---|---|
| *Pseudomonas* species | 22 (14.2) | 18(18.9) | 10 (22.7) |
| *Staphylococcus* species | 11 (7.1) | 5 (5.3) | NR |
| *Bacillus* species | 20 (12.9) | 16 (16.8) | 14 (31.9) |
| *Klebsiella* species | 9 (5.8) | NR | NR |
| *Streptococcus* species | 7(4.5) | NR | NR |
| *Micrococcus* species | 15 (9.7) | 11 (11.6) | 10 (22.7) |
| *Proteus* species | 9 (5.8) | NR | NR |
| *Citrobacter* species | 8(5.2) | 6 (6.3) | NR |
| *Enterobacter* species | 6(3.9) | 5 (5.3) | NR |
| *Corynebacterium* species | 13 (8.3) | 10 (10. 5) | NR |
| *Arthrobacter* species | 16 (10.3) | 13 (13.7) | NR |
| *Flavobacterium* species | 19 (12.3) | 11 (11.6) | 10 (22.7) |
| Total | 155 | 95 | 44 |

Key: NR= Not Recovered/Isolated. Numbers in brackets are percentages

Table 5: Microscopic, morphology and cultural characteristics of fungi isolated from the samples (non contaminated soil, contaminated soil and raw POME)

| Organisms | Types of Organisms | Microscopy morphology | Macroscopy morphology |
|---|---|---|---|
| *Aspergillus* species | Filamentous mold | Presence of septate hyphae; black long, smooth, erect conidiophores. Hyalineichotomously branched vesicle, round, radiate head. | Creamy to brownish-black mycelium with dark spores on the surface and often appears golden on the reverse side. |
| *Candida* species | Ovoid sphere yeast-like | Single clusters of blastoconidia which is round and elongate. Long branched pseudohyphas were also observed | Creamy to yellowish colonies with smooth, pasty, glistening or dry, wrinkled and dull color |
| *Penicillium* species | Filamentous mold | Presence of red pigment with edges surrounded by whitish margin. Also the conidiophores are branched. Septate and fruity mycelium are observed | A bluish-green filament is seen which changes to powdery greenish brown. Has brush phialospores arrangement |
| *Mucor* species | Filamentous mold | Presence of visible spore and short sporangiosphores with non-septate hyphae | A slimy colonies texture with dark pigmented spores. |
| *Fusarium* species | Filamentous mold | Presence of dark pigment of micro- and macro conidiophores and spherical in shape | Presence of sickle shaped macroconidia that is yellow to purple in colour. |

Out of the 95 isolates obtained, *Pseudomonas* species (18.9%) recorded the highest bacterial isolates from contaminated soil, followed by *Bacillus* species (16.8%), *Micrococcus* species (11.6%), *Flavobacterium* species (11.6%), while *Staphylococcus* species (5.3%) and *Enterobacter* species (5.3%). A total of 44 bacteria were obtained from Raw POME, *Bacillus* species (31.9%), recorded the highest followed by *Pseudomonas* species (22.7%), *Micrococcus* species (22.7%) and *Flavobacterium* species (22.7%).

Result of fungal identification showed that *Aspergillus* species, *Candida* species, *Penicillium* species, *Mucor* species and *Fusarium* species were identified (Table 5).

It was observed that there was increase in conductivity (μ/cm), oil and grease (mg/kg), total organic carbon, phosphates and nitrates in POME contaminated soils with the values: 88.05(μ/cm), 21.76 (mg/kg), 45.62 (mg/kg), 8.44 (mg/kg) and 9.26 (mg/kg) when compared with non contaminated soil samples with the values: 66.15 (μ/cm), 7.81(mg/kg), 23.06 (mg/kg), 3.11(mg/kg) and 5.15(mg/kg) respectively. But there was increase in sulphate and pH in non contaminated soil with values: 25.72 mg/kg and 6.42 when compared with POME contaminated soil with value 13.75 mg/kg and 5.25. It was also observed that there was increase in the exchangeable cations (Na, K, $Ca^{2+}$ and $Mg^{2+}$) in POME contaminated soil with values: 184.25 mg/kg, 7.36 mg/kg, 123.22 mg/kg and 1.87 mg/kg when compared with non contaminated soil (control) with values, 114.44 mg/kg, 2.94 mg/kg, 86.77 mg/kg and 1.44 mg/kg respectively as shown in Table 6.

Table 6: Physicochemical characteristics of the samples from oil palm mill effluent contaminated soil and non contaminated soil from Abakaliki L.G.A

| Characteristics | Non-POME contaminated soil | POME Contaminated soil |
|---|---|---|
| pH | 6.42 | 5.25 |
| Conductivity (μ/cm) | 66.15 | 88.05 |
| Oil and grease (mg/kg) | 7.81 | 21.76 |
| Total organic carbon (%) | 23.06 | 45.62 |
| Phosphate (mg/kg) | 3.11 | 8.44 |
| Nitrate (mg/kg) | 5.15 | 9.26 |
| Sulphate (mg/kg) | 25.72 | 13.75 |
| Sodium (mg/kg) | 114.4 | 184.25 |
| Potassium (mg/kg) | 2.94 | 7.36 |
| Calcium (mg/kg) | 86.77 | 123.22 |
| Magnesium (mg/kg) | 1.44 | 1.87 |

Table 7: Hydrocarbon-utilizing bacteria load of the non POME contaminated soil

| | Bacteria load of the sample ($X10^3$ cfu/g) | | | | |
|---|---|---|---|---|---|
| Location | Abakaliki | Ebonyi | Ikwo | Ishielu | Ohaukwu |
| Site 1 | 2.4 | 2.5 | 2.6 | 3.2 | 2.7 |
| Site 2 | 2.0 | 3.0 | 2.0 | 2.5 | 2.2 |
| Site 3 | 2.1 | 2.1 | 1.9 | 2.1 | 2.4 |
| Average | 2.2 | 2.5 | 2.2 | 2.6 | 2.4 |

Table 8: Hydrocarbon-utilizing bacteria load of the POME contaminated soil

| | Bacteria load of the sample ($X10^3$ cfu/g) | | | | |
|---|---|---|---|---|---|
| Location | Abakaliki | Ebonyi | Ikwo | Ishielu | Ohaukwu |
| Site 1 | 4.1 | 3.7 | 4.6 | 3.8 | 2.9 |
| Site 2 | 3.9 | 3.6 | 4.4 | 4.7 | 3.5 |
| Site 3 | 2.8 | 4.5 | 3.5 | 3.0 | 3.9 |
| Average | 3.6 | 3.9 | 4.2 | 3.8 | 3.4 |

Table 9: Hydrocarbon-utilizing bacteria load of the raw oil palm mill effluent

| | Bacteria load of the sample ($X10^3$ cfu/g) | | | | |
|---|---|---|---|---|---|
| Location | Abakaliki | Ebonyi | Ikwo | Ishielu | Ohaukwu |
| Site 1 | 1.4 | 1.2 | 2.0 | 1.1 | 1.6 |
| Site 2 | 1.3 | 1.3 | 1.0 | 1.5 | 1.4 |
| Site 3 | 1.1 | 1.2 | 1.3 | 1.3 | 1.1 |
| Average | 1.3 | 1.2 | 1.4 | 1.3 | 1.4 |

Table 10: Hydrocarbon utilizing-fungal load of the non-POME contaminated soil

| | Fungal load of the sample ($X10^3$ cfu/g) | | | | |
|---|---|---|---|---|---|
| Location | Abakaliki | Ebonyi | Ikwo | Ishielu | Ohaukwu |
| Site 1 | 2.1 | 1.5 | 2.0 | 1.7 | 2.0 |
| Site 2 | 2.7 | 1.7 | 2.8 | 2.3 | 1.8 |
| Site 3 | 2.2 | 2.0 | 2.5 | 2.0 | 1.7 |
| Average | 2.3 | 1.7 | 2.4 | 2.0 | 1.8 |

Table 11: Hydrocarbon utilizing-fungal load of the POME contaminated soil

| | Fungal load of the sample ($X10^3$ cfu/g) | | | | |
|---|---|---|---|---|---|
| Location | Abakaliki | Ebonyi | Ikwo | Ishielu | Ohaukwu |
| Site 1 | 3.0 | 3.3 | 2.9 | 3.2 | 3.0 |
| Site 2 | 2.8 | 3.1 | 3.5 | 3.5 | 3.8 |
| Site 3 | 2.5 | 2.7 | 3.0 | 2.3 | 2.9 |
| Average | 2.8 | 3.0 | 3.1 | 3.0 | 3.2 |

Table 12: Hydrocarbon utilizing-fungal load of the raw oil palm mill effluent

| | Fungal load of the sample (X$10^3$ cfu/g) | | | | |
|---|---|---|---|---|---|
| Location | Abakaliki | Ebonyi | Ikwo | Ishielu | Ohaukwu |
| Site 1 | 1.0 | 1.1 | 1.3 | 1.2 | 1.2 |
| Site 2 | 1.3 | 1.0 | 1.4 | 2.0 | 1.4 |
| Site 3 | 1.1 | 1.2 | 1. .6 | 1.5 | 1.5 |
| Average | 1.1 | 1.1 | 1.4 | 1.6 | 1.4 |

Most of the physicochemical parameters (conductivity, 88.05µ/cm; oil and grease, 21.76 mg/kg; total organic carbon, 45.62 %; Phosphate, 8.44 mg/kg; Nitrate, 9.26 mg/kg; Sodium 184.25 mg/kg; Potassium, 7.36 mg/kg; Calcium, 123.22 mg/kg and Magnesium, 1.87 mg/kg) were higher in POME contaminated soil than in non-contaminated soil of oil palm mill (conductivity, 66.15 µ/cm; oil and grease, 7.81 mg/kg; total organic carbon, 23.06 %; Phosphate, 3.11 mg/kg; Nitrate, 5.15 mg/kg; Sodium, 114.4 mg/kg; Potassium, 2.94 mg/kg; Calcium, 86.77 mg/kg and Magnesium, 1.44 mg/kg), but some physicochemical parameters were higher in non-contaminated soil (pH, 6.42 and sulphate, 25.72 mg/kg) than in POME contaminated soil (pH, 5.25 and sulphate, 13.75 mg/kg) as indicated in Table 6.

Result of the hydrocarbon-utilizing bacteria load of the samples showed that in non contaminated soil (control); Ishielu L.G.As has the highest hydrocarbon-utilizing bacteria load with value of 2.6x$10^3$ cfu/g, followed by Ebonyi L.G.A with value of 2.5x$10^3$ cfu/g, then Ohaukwu L.G.A with value of 2.4x$10^3$ cfu/g and the lowest hydrocarbon utilizing bacteria load was seen in Abakaliki and Ikwo L.G.A with value of 2.2x$10^3$ cfu/g as shown in Table 7.

For the contaminated soil, Ikwo L.G.A has the highest hydrocarbon utilizing bacteria load with value of 4.2x$10^5$cfu/g followed by Ebonyi L.G.A with value of 3.9x$10^5$ cfu/g, then Ishielu L.G.A with value of 3.8x$10^5$ cfu/g and Abakaliki L.G.A with value 3.6x$10^5$ cfu/g, the lowest hydrocarbon-utilizing bacteria load was seen in Ohaukwu L.G.A with value of 3.4x$10^5$ cfu/g as indicated in Table 8.

For the raw oil palm mill effluent, the highest hydrocarbon-utilizing bacteria load was seen in Ikwo and Ohaukwu L.G.A with value of 1.4x$10^2$ cfu/ml, followed by Abakaliki and Ishielu L.G.A with value of 1.3x$10^2$ cfu/ml and the lowest bacteria load was seen in Ebonyi L.G.A with value of 1.2x$10^2$ cfu/ml (Table 9).

Result of the hydrocarbon-utilizing fungal load in non POME contaminated soil (control) shows that Ikwo L.G.A has the highest hydrocarbon-utilizing fungal load with value of 2.4x$10^3$ cfu/g, followed by Abakaliki L.G.A with value 2.3x$10^3$ cfu/g, followed by Ishielu L.G.A with value of 2.0x$10^3$ cfu/g, then followed by Ohaukwu L.G.A with value 1.8x$10^3$ cfu/g and the lowest hydrocarbon-utilizing fungal load was seen in Ebonyi L.G.A with value 1.7x$10^3$ cfu/g (Table 10).

The highest hydrocarbon-utilizing fungal load in the POME contaminated soil was seen in Ohaukwu L.G.A with value 3.2x$10^4$ cfu/g, followed by Ikwo L.G.A with value of 3.1x$10^4$ cfu/g, then Ebonyi and Ishielu L.G.A with value of 3.0x$10^4$ cfu/g and the lowest hydrocarbon utilizing-fungal load was seen in Abakaliki L.G.A with value 2.8x$10^4$ cfu/g (Table 11).

The highest hydrocarbon-utilizing-fungal load in the raw palm oil effluent was seen in Ishielu L.G.A with average of 1.6x$10^2$ cfu/ml, followed by Ikwo and Ohaukwu L.G.A with value of 1.4x$10^2$ cfu/ml and the lowest hydrocarbon-utilizing fungal count was seen in Abakaliki and Ebonyi L.G.A with value of 1.1x$10^2$cfu/ml as shown in Table 12.

## DISCUSSION

Direct release of crude industrial wastewater may have great influence on the physicochemical and biological characteristics of the soil. The level of pollutant from POME differs with the quality of the raw material and production process used to produce the palm oil [24]. Result of this present study revealed that bacteria load of the samples revealed that the non contaminated soil (control) has the highest bacterial load, while the raw palm oil mill recorded the least in all the sample locations studied as shown in Table 1. In line with report of this work, Okpokwasili and James [25] revealed that the lower counts recorded in the POME may be attributed to its acidic and oily content as only microorganisms with the competent enzyme systems to proliferate can be found in it. Although POME contains metabolizable nutrients, the high concentration of POME at the dump site, together with excess water suppressed the growth of the organisms. The excessive moisture may have created anaerobic conditions [26]. This study is contrary to study of Eze, Okwulume and Agwung [27] who reported that the control soil had the lowest microbial counts since it is devoid of POME which enriched the polluted soil over time. The international standards for drinking water states that potable water should not contain 100 cells of Total Heterotrophic Bacteria per 100 ml of water but unfortunately, the bacterial counts obtained in the POME from both factories exceeded the standard (WHO, 1993) which could pose threat to public health causing gastrointestinal diseases when discharged water bodies. The toxicity of POME may also be due to the presence of phenols and other organic acids which are responsible for its phytotoxic effect and antibacterial activity [28, 29].

Fungi are notably aerobic and can also grow under environmentally stressed conditions such as low pH and poor nutrient status [30]. These are conditions which were brought about in the POME polluted soil by the properties of POM [31]. The fungal load of the samples revealed that the non contaminated soil (control) has the highest bacterial load, followed by the contaminated soil and the raw palm oil mill effluent recorded the lowest in all the sample locations studied as shown in Table 2. In the same vein the control had greater numbers of microorganisms than the POME contaminated soil in all the groups of microorganisms in the different seasons [32]. Results showed that soils where palm oil mill effluents (POME) were freshly discharged have very scanty microbial population and diversity [33].

Result of the morphological and biochemical characteristics of the bacterial isolates showed that *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species, *Proteus* species, *Citrobacter* species, *Streptococcus* species, *Flavobacterium* species, *Arthrobacter* species, *Micrococcus* species, *Corynebacterium* species, *Enterobacter* species *and Klebsiella* species were isolated from the non contaminated (control) soil samples while *Bacillus* species, *Pseudomonas* species, *Staphylococcus* species, *Citrobacter* species, *Flavobacterium* species, *Arthrobacter* species, *Micrococcus* species, *Corynebacterium* species and *Enterobacter* species were isolated from the soil contaminated with oil palm mill effluents. The organisms isolated from the raw oil palm mill effluents include species, *Pseudomonas* species, *Bacillus* species, *Flavobacterium* species and *Micrococcus* species (Table 3). The bacteria genera found are in line with the previous reports [34 - 37]. The genera identified are wide spread and many of the individual species have been shown to be able grow on petroleum hydrocarbon [38-40].

*Pseudomonas* species was recorded the highest bacterial species obtained from non-contaminated soil (14.2%) and contaminated soil (18.9%), while raw POME recorded *Bacillus* species (31.9%) as the highest bacteria species as shown in Table 4. [41] reported that the most predominant hydrocarbon degrading bacteria found in POME contaminated soil and POME belong to the following genera *Pseudomonas*, *Bacillus*, *streptococcus*, *Citrobacter*, *Staphylococcus*, *Klebsiella* and *Enterobacter*. The isolation of species of *Pseudomonas, Bacillus* and *Proteus* species in POME amended soil had previously been reported [42, 43]. The occurrence of *Pseudomonas aeruginosa* in POME polluted soil may be due to their ability to utilize oil as their carbon source [44]. Pseudomonads are the best known bacteria capable of utilizing hydrocarbons as carbon and energy sources and producing biosurfactants when grown on carbon sources [45]. WHO [46] reported *Pseudomonas species*, *Serratia* species, *Bacillus* species, *Staphylococcus* species and *Corynebacterium* species from small holder oil palm mill processing mills in Nigeria.

The fungal genera identified were *Aspergillus* species, *Candida* species, *Penicillium* species, *Mucor* species and *Fusarium* species were identified as shown in Table 5. These were in line with works of Paredes *et al.* [34]; Pascual *et al.* [35] , Singer and Finnerty [40], Zakaria, Hazon and Mwide [47]. 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 Eze and Okpokwasili [9] who noted that the nature and behavior of the microbial population in the POME environment are influenced by many physio-chemical parameters of ecological importance. *Aspergillus* species in particular are reported to be good producers of cellulases; the enzymes responsible for the breakdown of cellulose in POME [17]. The occurrence of moulds particularly of the genera in *Aspergillus*, *Penicillium*, *Mucor* and *Fusarium* in POME contaminated soil has been reported by several authors [6-9].

Most of the physicochemical parameters (conductivity, 88.05µ/cm; oil and grease, 21.76 mg/kg; total organic carbon, 45.62 %; Phosphate, 8.44 mg/kg; Nitrate, 9.26 mg/kg; Sodium 184.25 mg/kg; Potassium, 7.36 mg/kg; Calcium, 123.22 mg/kg and Magnesium, 1.87 mg/kg) were higher in POME contaminated soil than in non-contaminated soil of oil palm mill (conductivity, 66.15

μ/cm; oil and grease, 7.81 mg/kg; total organic carbon, 23.06 %; Phosphate, 3.11 mg/kg; Nitrate, 5.15 mg/kg; Sodium, 114.4 mg/kg; Potassium, 2.94 mg/kg; Calcium, 86.77 mg/kg and Magnesium, 1.44 mg/kg), but some physicochemical parameters were higher in non-contaminated soil (pH, 6.42 and sulphate, 25.72 mg/kg) than in POME contaminated soil (pH, 5.25 and sulphate, 13.75 mg/kg) as indicated in Table 6. Increase in Calcium, total organic carbon, phosphate, sodium, nitrate, potassium and calcium content in POME contaminated soil in comparison with the non contaminated soil (control) showed improvement of the soil quality. Several Researchers observed similar results and attributed the increase to the addition of POME to the soil, hence, increase in exchangeable bases levels [23 - 25]. POME can be used as fertilizer or animal feed substitute in terms of providing sufficient mineral requirements because of its fertilizing property. It can be used by farmers when properly treated and packaged in rural and urban areas to improve soil fertility thereby increasing the agricultural productivity for global, national and regional food demands [40]. Result on the hydrocarbon-utilizing bacteria and hydrocarbon-utilizing fungi counts from the palm oil mill effluent contaminated soil (Table 8 and Table 11) when compared to the non-contaminated soil (Table 7 and Table 10) showed that There was increase in hydrocarbon utilizing microbial loads from the palm oil mill effluent contaminated soil (Table 8 and Table 11) when compared to non-contaminated soil (Table 7 and Table 10). This is in agreement with the findings of Huan [11], Ibe *et al.* [12] Cassida, Klein and Santoro [13]; Kittikun *et al.*[14], who reported gradual increase in microbial population in the palm oil mill effluent contaminated soil. This is understandable as oil palm mill degrading bacteria thrive well where there is abundance of the oil palm mill effluent substrate. The result of this study revealed that the microbial populations in POME contaminate soils have high potentials of mineralizing POME in the environment to safe and acceptable level. The lowest hydrocarbon utilizing microbial loads was obtained with the raw palm oil mill effluent (Table 9 and Table 12).

## CONCLUSION

From the report of this study, it is observed that POME could have a positive effect if discharged properly since little application of the effluent can enhance microbial proliferation which in return increases soil fertility. Hence, the government should create alertness to people involved in palm oil processing (both small and large scale) on the requirement for proper disposal POME or improve POME quality, because if not properly managed, it can negatively affect soil fertility by hindering microbial proliferation.

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