Studies on the microorganisms associated with Palm Oil Polluted Soils

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Abstract

Palm oil is an edible vegetable oil derived from the fruits of the oil palm tree. Spillage of palm oil on soil is unavoidable in the oil industry. This contamination or pollution has an impact on the soil's composition.This study was therefore aimed at identifying the microorganisms associated with palm oil-polluted soil, chaff and unpolluted soil samples (which served as control); and studying the effect of the oil pollution on the soil microflora. Samples obtained from two different sites (Oda farm, Akure and Ori eeru, Iwo) were used. Experiments were carried out using pour plate technique. Each sample was also screened for physicochemical tests. The pH range of the Oda farm and Ori eeru are 4.9-6.5 and 5.2-5.9 respectively. The moisture content of the Oda farm and Ori eeru samples are 33-95% and 28-39%. Bacteria isolated from the polluted soil include *Bacillus* sp, *Staphylococcus aureus*, *Proteus* sp, *Corynebacterium* sp and *Micrococcus* sp; while the fungi include *Aspergillus flavus, Aspergillus niger, Mucor* sp and *Rhizopus* sp. The higher bacterial and fungal counts of the polluted soil samples were 7.25 $x10^3$ and 6.05 x 10^3 cfu/ml respectively. The chaff had the highest fungal count of 13.0 cfu/ml of all the tested samples, while the highest bacterial population (20.7 $x10^3$) was recorded in the unpolluted soil. Most of the isolates are pathogenic species. It is essential that palm oil effluents are treated before disposal to avoid soil contamination and alteration of the soil microorganisms. Modernized methods should be employed in palm oil production to avoid spillage during processing.

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Introduction

Palm oil (*Elaeis guineensis*) is vegetable oil and ingredient in the diet of many people. According to MPOC (2007), it is the world's largest source of edible oil. Palm oil is a product extracted from the flesh of individual fruit contained on the bunch. Many people cultivate oil palm as a means of livelihood and a source of income. This is encouraged by the worldwide increase in the demand for palm oil. Oil palm is known and often referred to as a crop of multiple values, due to its high economic importance (Akangbe *et al*., 2011). The demands for domestic and industrial applications of palm oil have continued to increase (Omereji, 2005). It is estimated that for every Nigerian household of five, about two liters of palm oil are consumed weekly for cooking (Ekine and Onu, 2008). However, palm oil is an essential multipurpose raw material for both food and non-food industries (Armstrong, 1998).

Before 1965, Nigeria was the world's leading producer and exporter of palm oil and has since 1974 ceased to contribute to the export trade in the commodity, largely due to increased domestic demand that has not kept pace with the production (Omoti, 2004). During the past decade, Nigeria has become a net importer of palm oil (Olagunju, 2008). In 1985, palm oil production in Malaysia increased from 4.1 million tonnes to 6.1 in 1990. This projected to 19.4 million in 2012. Presently, Nigeria is the fifth-largest palm oil producer, though it ranks third in the world in terms of land planted with oil palm (Izah *et al.*, 2016). However, there is a plan to increase the palm oil production from 600,000 tons a year to 5 million tons a year by 2027. Indonesia is currently the world's largest producer of palm oil-producing over 29 million tonnes, this is followed by Malaysia with over 19 million tonnes, and Thailand and Colombia with 1.8 and 1.1 million tonnes respectively (Izah *et al.*, 2016). Palm oil is extracted from fresh fruit bunches by a mechanical process. An average size bunch weighs about 20-30 kg and contains 1500-2000 fruits. These

are harvested according to harvesting cycles and delivered to the mills on the same day. The quantity of crude palm oil is dependent on the care taken after harvesting, particularly on the handling of bunches. A palm oil mill produces crude palm oil and kernels as primary products and biomass as secondary products. A typical mill has many operation units which include sterilization, stripping, digestion and pressing, clarification, purification, drying and storage. For the palm kernel line, there are steps such as nut/ fibre separation, nut conditioning and cracking, cracked mixture separation and kernel drying, storage. The dried kernels are often sold to palm kernel crushers for the extraction of crude palm kernel oil.

However, palm oil production results in the generation of large quantities of polluted wastewater commonly as palm oil mill effluent. In the process of palm oil milling, the effluent is generated through sterilization of fresh oil palm fruit bunches, clarification of palm oil and effluent from hydrocyclone operations (Borja *et al*., 1996).

The chaff is extracted during palm oil processing. It is a solid waste from the process. It can be further processed into useful products and can also be used for burning.

This work identified the microorganisms present in palm oil-polluted soil in order to determine the organisms that are present in palm oil-polluted soil.

Materials and methods

Sampling Sites

Two different sites/locations were sampled; which are Oda farm (Akure, Ondo State) with latitude 7.16^{o}N and longitude 5.25^{o}E and Ori eeru (Iwo, Osun State) with latitude 7.64^{o}N and longitude 4.20^{o}E. On both sites, palm oil-polluted soil, the chaff and soil samples not polluted with palm oil which served as the control were collected.

Collection of Samples

Samples were collected in January, by removing litter or surface debris and at a depth of 5cm. The samples were poured into sterile jam bottles which were tightly closed, labeled appropriately with the date and location of sample collection.

Physiochemical Analysis

Determination of pH

Ten grams each of the palm oil-polluted soil, shaft and soil samples from the two different sites were weighed and transferred into separate clean beakers. Fifty milliliters (50ml) of distilled water was added. Each mixture was stirred using a glass rod for 10 minutes. The mixture was allowed to settle and the pH was determined using a pH meter (R1 02895: Woon socket, pocket-sized pH meter HANNA instrument).

Determination of Moisture Content

For each sample, a crucible was dried in the oven, allowed to cool in a desiccator and its weight recorded. Ten grams each were weighed into the crucible and the crucible was re-weighed. Each sample was dried in an oven at 105^{o}C for 24 hours. The crucibles were transferred into a desiccator and allowed to cool. The crucibles and the oven-dried samples were weighed and the weight of the dried samples was determined by subtraction. The samples were dried repeatedly at 12 hour-interval until a constant weight was obtained. The percentage of moisture in the samples was calculated using the following formula:

$$\frac{\text{Loss in weight of sample}}{\text{Initial weight of sample}} \times \frac{100}{1}$$

Determination of Total Organic Matter

Two grams of the oven-dried sample from the experiment above was weighed and transferred into a previously weighed crucible. The crucible was ignited over a Bunsen burner to bright red heat and the sample stirred occasionally with a steel rod. The crucible was heated for 15 minutes and was allowed to cool in the desiccator. The weight of the crucible was recorded thus, the loss in weight by difference was obtained. The percentage of total organic matter in the sample was calculated using the following formula:

$$\frac{\text{Loss in weight of ignited sample}}{\text{Weight of dried sample}} \times \frac{100}{1}$$

Sterilization techniques

During this experiment, glasswares used such as conical flask were washed properly before the medium was prepared and afterward autoclaved at 121^{o}C and pressure of 1.0kg/cm^{2} for 15 minutes.

Preparation of Culture medium

Nutrient agar was used in culturing the isolates. The medium was prepared according to the manufacturer's specification; and was sterilized at 121^{o}C and pressure of 1.0kg/cm^{2} for 15 minutes.

Isolation Procedures

The organisms were isolated using the pour plate method, allowed to solidify before incubation at 37^{o}C for 24 hours. Pure cultures of isolates were obtained by streaking and were later used for further analysis.

Identification of pure isolates

The pure bacterial cultures were identified by the cultural (cell shape, colour, shape) and various and biochemical (MRVP, citrate, indole, starch hydrolysis,

sugar fermentation) tests. Fungal isolates were observed under the microscope at a magnification 400.

Results

Figures 2, 3, 4 and 5 show the bacteria population that were present in the palm oil-polluted soil, chaff and unpolluted soil sample from Oda farm and Ori eeru. For the Oda farm samples, bacterial counts in the palm oil-polluted soil, chaff and unpolluted soil samples are 5.4×10^3cfu/ml, 8.15×10^3cfu/ml and 3.35×10^3cfu/ml while the fungal counts are 6.05×10^5cfu/ml, 9.45×10^5cfu/ml and 5.15×10^5cfu/ml respectively. For the Ori eeru samples, bacterial counts in the palm oil-polluted soil, chaff and unpolluted soil samples are 7.25×10^3cfu/ml, 5.85×10^3cfu/ml and 20.7×10^3cfu/ml while the fungal counts are 5.0×10^4cfu/ml, 13.0×10^4cfu/ml and 8.0×10^4cfu/ml respectively.

Table 1 shows the physicochemical properties of the palm oil-polluted soil, chaff and unpolluted soil samples from Oda farm, Akure and Ori eeru, Iwo. The pH values of the palm oil polluted soil, chaff and unpolluted soil sample of Oda farm and Ori eeru are 4.9, 5.9, 6.5 and 5.2, 5.7 and 5.9 respectively. For the Oda farm samples, the moisture content of the palm oil polluted soil, chaff and unpolluted soil samples are 93, 33 and 45 while for the Ori eeru samples, the moisture content are 35, 28 and 39 respectively. For the Oda farm samples, the organic matter of the palm oil polluted soil, chaff and unpolluted soil samples are 50, 65 and 5 while for the Ori eeru samples, the organic matter are 55, 75 and 15 respectively. *Corynebacterium* sp, *Micrococcus* sp, *Bacillus cereus*, *Bacillus coagulans*, *Proteus* sp, *Staphylococcus aureus*, *Pseudomonas* sp and *Klebsiella pneumoniae* were identified as bacterial isolates from Oda farm.

From Ori eeru site, the bacteria include *Corynebacterium* sp, *Micrococcus* sp, *Bacillus* sp, *Bacillus pumulis*, *Proteus* sp, *S. aureus* and *E. coli*. Fungi identified from from Oda farm and Ori eeru are *Rhizopus* sp, *Aspergillus niger*, *Aspergillus flavus*, *Mucor* sp. and *Rhizopus* sp, *Aspergillus niger*, and *Mucor* sp. respectively. Plates 6-9 shows the identification of fungi under photomicrograph.

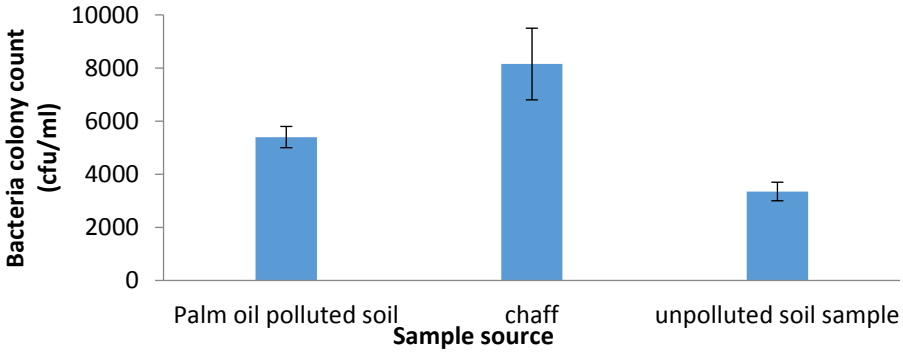

Figure 1: Bacterial population of the samples from Oda farm: Error bar indicates standard deviation

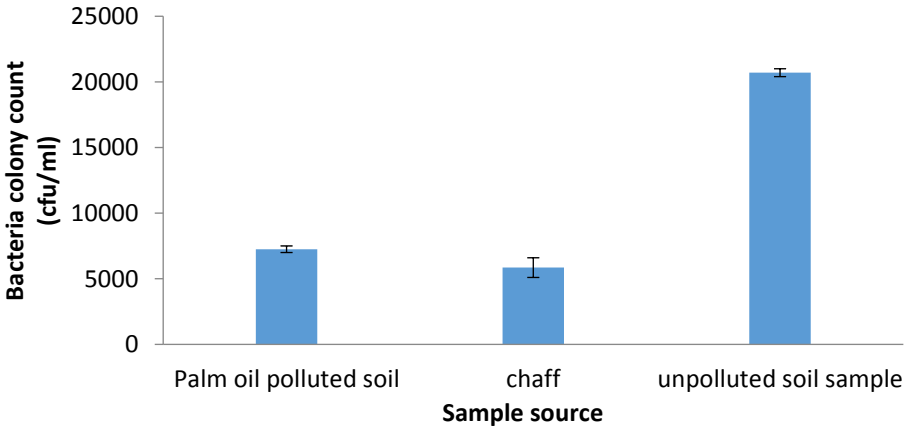

Figure 2: Bacterial population of the samples from Ori eeru: Error bar indicates standard deviation

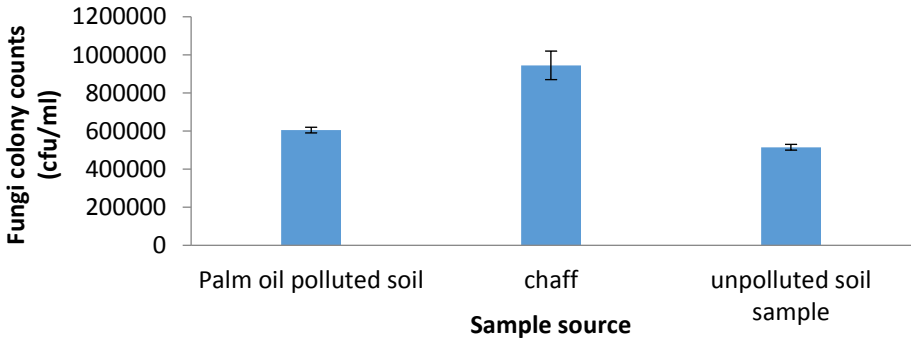

Figure 3: Fungal population of the samples from Oda farm: Error bar indicates standard deviation

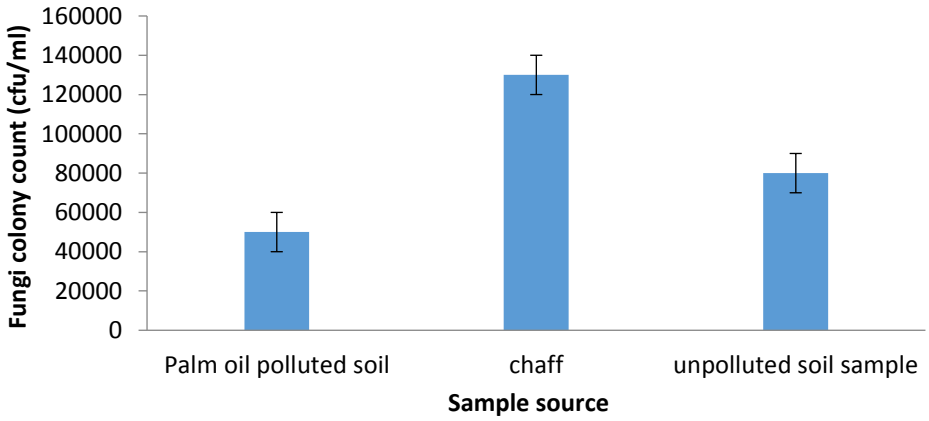

Figure 4: Fungal population of the samples from Ori eeru; Error bar indicates standard deviation

Table 1: Physiochemical Properties of The Samples

Sample	Ph	% Moisture content	% Organic Matter
A	4.9	93	50
B	5.9	33	65
C	6.5	45	5
D	5.2	35	55
E	5.7	28	75
F	5.9	39	15

Keys:
ODA FARM- SITE 1; Sample A: Palm oil polluted soil; Sample B: Chaff; ;Sample C: Unpolluted soil sample (control)
ORI EERU- SITE 2; Sample D: Palm oil polluted soil;Sample E: Chaff;Sample F: Unpolluted soil sample (control)

Discussion

Results from this research have shown that the pH of the palm oil polluted soil from both sites were acidic relative to the unpolluted soil sample (control). The pH values of the polluted, chaff and unpolluted soils sites, Oda farm and Ori eeru were 4.9 and 5.2; 5.9 and 5.7; and 6.5 and 5.9 respectively. The observation correlates with the report of Lam and Lee (2011), who reported acidity in the palm oil polluted soil collected from a palm oil industry in Malaysia. Yossan and Prasetsan (2012) also reported an initial pH of 4.5 to 6.5 in palm oil mill effluent which also affects the hydrogen production with optimum pH of 6.0. Acidity bacteria in the palm oil polluted soil is due to presence of organics and the high level of some organic acids in the effluents (Nmaduka, 2018). The pH range of the samples supported the growth of both bacteria and fungi. Bacteria are known to grow best around neutral pH values (6.5 and 7.0), but some can tolerate acidic conditions (even pH as low as 1.0). Fungi thrive at slightly acidic conditions with pH values of 5.0 and 6.0. This therefore explains the microbes that were isolated in this study.

The moisture content of the palm oil polluted soils were relatively high (93% and 35%), compared to the chaff (33% and 28%). However, the moisture content of the unpolluted soil (control) was higher (45% and 39%) than that of the chaff samples for both sites. The moisture content of 93% of site 1 could be due to since that the effluent is more than what was observed on the other sampling site. There was also a similarity in the level of organic matter in the soil samples. Generally, the chaff had the highest percentage of organic matter (65% and 75%) for both sites, This could be due to the presence of more ions in the soil as a result of the continuous deposit of palm oil on such soils. This was followed by the polluted samples (50% and 55%). The least organic content was recorded in the unpolluted soils (5% and 15%). In comparison, Chin *et al.* (1996) have reported that palm oil mill effluent contains a high concentration of organic matter in relation to uncontaminated soils. Palm oil mill effluent contains a large percentage of the degradable organic matter probably due to the presence of uncovered palm oil. It is a measure of the organic carbon concentration in the soil and is responsible for the improvement of the structure and the water holding capacity of the soil (Nmaduka, 2018).

The bacteria isolated from all the samples were *Micrococcus* species, *Bacillus* sp, *Proteus* sp, *E. coli*, *K. pneumoniae*, *Corynebacterium* sp, *Pseudomonas* species and *S. aureus*. All these except *E. coli* and *K. pneumoniae* were isolated from the polluted soil samples. This indicates that these samples are sources of pathogenic bacteria that are capable of contaminating the produce. Eze *et al*. (2013) isolated *Bacillus* species and *Proteus* species amongst other bacteria from palm oil polluted soils. According to Dominika (2015), the presence of *Proteus* sp in palm oil polluted soil is due to the ability to tolerate or utilize polluting compounds. Also, according to Mohammadreza and Soheila (2014) the isolated *Bacillus* may be as a result of the ability of the bacterium to tolerate or grow in oily environment. *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Proteus*, and *Micrococcus* species were also reported as isolates of vegetable oil polluted soil by Popoola and Onilude (2017).

The fungi encountered in the palm oil polluted soil in this study include *A. niger*, *Mucor* species, and *Rhizopus* species. Similar results were reported by Eze *et al*. (2013) and Mohammadreza and Soheila (2014) who isolated *Rhizopus* species; *Aspergillus* species and *Mucor* species respectively from the soil samples. The presence of *Mucor* sp. in the palm oil polluted soil shows that the fungus is can survive in a hostile environment (Nwuche and Ogbonna, 2011; Ohimain *et al*., 2012a; Ohimain *et al*., 2012b). In contrast, Mohammadreza and Soheila (2014) isolated *A. fumigatus*, *Candida* species, *Fusarium* species, and *Penicillium* species from palm oil mill effluent. Eze *et al*. (2013) also isolated *Geotrichum* species, *Trichoderma* species, *Fusarium* species, and *Penicillium* species from palm oil mill effluent, while Popoola and Onilude (2017) isolated *Penicillium*, *Candida*, *Geotrichum*, *Saccharomyces*, *Kluveromyces.*

The bacterial load of the unpolluted soil sample from Ori eeru was the highest among all the tested samples; while it was the least in Oda farm samples. It is worth noting that the bacteria load of palm oil polluted soil from both sites was the intermediate, neither the highest nor the least. The population of the fungi associated with the chaff from both experimental sites were high and even the highest amongst other tested samples (9.45×10^5 and 13×10^4 cfu/ml). Bacterial count from the polluted soil samples were 5.4 and 7.25×10^3 cfu/ml, while for the unpolluted soil, the count was relatively high (20.7×10^3 cfu/ml), for one of the sites and very low (3.35×10^3 cfu/ml) in the other. The chaff also had an appreciable count of bacteria; 8.15 and 5.83×10^3 cfu/ml. Fungal count of the polluted soil was higher than that of the unpolluted soils from the two sites; 6.05×10^5 and 5.0×10^4 as compared to 5.15×10^5 and 8.0×10^4 cfu/ml. This result is an indication

that palm oil polluted samples, especially chaff are good sources of fungi.

Conclusion

The waste from palm oil mills pollutes the environment and serves as a fertile breeding ground for a wide range of microorganisms. A large number of these bacteria can survive in an oily environment because they produce lipase enzyme or spores. When the oil is contaminated by pathogenic microbes, it poses a health risk for its consumers. Before effluents are released into the environment, proper waste treatment is required. The use of more modern methods of palm oil production will help to keep oil from leaking out during processing.

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