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Effects of Palm Oil Processing Waste on Soil Microorganisms

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Abstract

This work was carried out to perform the comparative analysis on the effects of palm oil processing waste (POPW) on soil Microorganisms. Three soil samples were collected from three different locations, North bank, Wurukum, and High-level where palm oil processing waste are being discharged. The samples were analyzed, Soil samples unimpacted with POPW served as control. The physicochemical parameters of the soil samples that are pH, moisture, Temperature and particulate nature were analysized using standard methods. The total coliform count, total viable count and total fungal counts were evaluated using the pour plate method. Result showed that the Total Coliform, Total viable count and Total fungal counts (200.77 ±16.525 mean of the three samples, 22.55 ±2.041 mean of three samples and 28.88 ±2.590 mean of the three samples) of the impacted soils were lower compared to the values (291.00 ±13.00 in control soil, 118.33 ±18.717 control soil and 44.33 ±1.527 control soil respectively) of the un-impacted soils which served as control. The bacterial genera isolated from these soil samples include; Bacillus spp, Klebsiella, Staphylococcus spp, Salmonella spp and Proteus spp. The fungal genera isolated from soils with POPW includes; Aspergillus spp, Rhizopus spp, and Mucor spp, the results obtained indicates that POPW have immediate negative impact on soil microbiota as their application could lead to changes which affects soil microbial communities. Therefore, it would be advised that proper guidelines be set up for possible pre-treatment and safe discharge of POPW, in other to avoid its effect on soil micro biota and soil fertility.

Keywords: Soil; Palm Oil; Bacteria; Fungi; Waste Water

Abbreviations: POPW: Palm Oil Processing Waste; NA: Nutrient Agar; SDA: Sabouraud Dextrose Agar.

Introduction

Oil palm, (*Elaeis guineensis*) African tree in the palm family (*Arecaceae*), cultivated as a source of oil. The oil palm is grown in West and Central Africa, as well as in Malaysia and Indonesia. Palm oil, obtained from the fruits, is used in making soaps, cosmetics, candles, biofuels, and lubricating greases and in processing tinplate and coating iron plates. Palm kernel oil, from the seeds, is used in manufacturing such edible products as margarine, chocolate confections, cookies, and bread, as well as many pharmaceuticals. The cake residue after kernel oil is extracted is use as cattle feed. The plant is also grown as an ornamental in many subtropical areas [1]. The global production of palm oil is increasing rapidly, which is leading to an increase in waste from palm oil mills. This waste is causing significant environmental problems [2]. Soil is the key component of natural ecosystem [3], and as such environmental sustainability depends largely on a sustainable soil ecosystem. Soil can be defined as a dynamic, living ecosystem that performs a variety of functions, including supplying nutrients and water to plants, storing and cycling carbon and other elements, and providing habitat for a vast array of microorganisms and invertebrates.

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The soil is composed of five components: minerals, water, soil air, organic matter and soil living organisms. Due to its chemical composition and physical properties soil form a habitat for larger amounts of microorganisms and other living organisms [4]. Microorganisms found in the soil include bacteria, algae, fungi, actinomycetes, protozoa and viruses, but bacteria are the most abundant of all soil microorganisms. They are characterized by high metabolic activity. Most soil bacteria are characterized by the ability to adhere to surfaces of the mineral molecules and to the soil colloids [5].

While human industrial and economic activities have improved living standards in many ways, they have also led to increased waste production. This waste is often disposed of on land, which can disrupt the natural ecological balance and pose a threat to human health and the survival of microorganisms in the environment [6]. Palm oil processing waste (POPW) is one of the major most problematic environmental pollution potentials among wastes [7]. It has the residual liquid waste product obtained after the extraction of oil from the fruits of the oil palm. It consists of the liquid effluent arising from the sterilization of the fruits (the sterilizer condensate) and the sludge effluent (clarification sludge) arising from the maceration of the fruit mesocarp and subsequent decantation/clarification of the oil. Various methods have been proposed for disposing of palm oil waste, including both traditional and innovative solutions [3]. Some methods of palm oil waste disposal include channeling the waste into ditches, landfills, and streams and rivers, or simply discharging it onto open land. However, these methods can have negative environmental and health impacts. In Nigeria, palm oil extraction is often carried out by small-scale farmers using semi-mechanized methods. This results in the production of large amounts of waste, which is often dumped on nearby land without any further processing [8]. When the palm oil waste has accumulated considerably in the area due to continued deposit, the site is abandoned and a fresh site is located. The negative environmental effects of palm oil waste being discharged into streams have been well documented. However, the impact of palm oil waste on soil microorganisms has not been studied in depth. This is an important issue that needs to be addressed, as soil microorganisms play a vital role in maintaining soil health and fertility [9]. The aim of this research is to determine the effect of Palm oil processing waste on soil microorganisms.

Materials and Methods

Study Area

This study was carried out within Makurdi metropolis, the capital of Benue state. Makurdi is the Administrative headquarters of Benue State lies approximately between latitudes 7°39' and 7°45'N and longitudes 8°33' and 8°35'E. The town is located along the coast of River Benue. The climatic condition in Makurdi is influenced by two air masses: the warm, moist south westerly air masses and the warm dry north-easterly air masses. The south-westerly air mass is a rain-bearing air mass that brings about rain from the month of May to the month of October while the dry north-easterly air mass blow over the region from November to April brings about seasonal drought. The mean annual rainfall in Makurdi is about 1290mm. Temperature generally in Makurdi is high with February and March as the hottest months. Temperature in Makurdi varies from a daily of 40°C and a minimum 22.5°C. Makurdi town like most cities in lower Benue valley is drained by the Benue River and its tributaries. Due to the general low relief of Makurdi, stabilize portion of the area is waterlogged and flooded during heavy rainfall [10].

Sample Collection

Soil samples from Palm oil processing site were collected from three different locations of local palm oil mill site in Makurdi Benue state, Nigeria. Soil samples of 0-15cm depth were collected from the site by excavation using trowel, another soil samples was collected far from the oil processing site which served as control. The collected samples from all locations were thoroughly mixed on the spot in order to obtain an even distribution. The samples were air-dried and sieved using a 2 mm sieve and then 5kg of the soil was be weighed and stored in six fresh, clean polyethylene bags prior to laboratory analysis so as to maintain the stability of samples without significant alteration in their biological properties.

Sterilization and Disinfection of Materials to be Used

All the glass wares to be used during the bench work were properly washed with detergent, rinsed with clean water and sterilized in hot air oven at 160oc for 1hour. Also, wire loop was sterilized by flaming on the Bunsen burner to red hot before inoculation. The work bench was properly disinfected before and after any work.

Preparation of Media for Microbial Analysis

The media used for isolation of microorganisms include nutrient agar, MacConkey agar, salmonella shigella agar, blood agar, mannitol salt agar, nutrient broth, Sabouroud Dextrose agar and potato dextrose agar. The different media used in Isolation was prepared according to manufacturer's instructions.

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Microbiological Analysis of the Samples

Microbial analysis was carried out on the soil samples. 10 g of soil sample was collected aseptically and labeled. Microbial analysis was carried out within 24 hours of sampling so as to maintain the stability of the sample without significant alteration in the microbial population. Serial dilution was carried out by weighing 10 g of soil in to 90 ml of sterile saline water contained 200 ml volumetric flask and agitated to dislodge the microorganisms from the soil particles. From this initial dilution, a ten-fold serial dilution was prepared.

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 will be incorporated with antifungal agent (50 µg/ml Nystatin), in order to prevent the growth of fungal contaminants. Bacterial colonies were counted after 24 hours of incubation at room temperature and reported as colony forming units (cfu/g) of soil [3]. Bacterial colonies were repeatedly transferred to freshly prepared nutrient agar plates by the streak-plate method and allowed to grow for 48 hours before stocking.

Fungi: The total heterotrophic fungi count was measured by pour plating 1 ml of 10^{-3}dilution into Sabouraud Dextrose Agar (SDA) supplemented with antibacterial agents (50 µg/ml of streptomycin and 30 µg/ml of penicillin) to inhibit the growth of bacterial contaminants. Fugal counts were reported after 72 hours of incubation [11]. Similarly, distinct fungal colonies were sub-cultured on freshly prepared Sabouraud Dextrose Agar plates. Pure isolates of the microorganisms will be maintained on agar slants as stock, which will be preserved in the refrigerator for further use.

Identification and Isolation of Microorganisms from Collected Samples

Bacteria and fungi were isolated and characterized using cultural identification, morphological identification, using Gram staining reaction and other biochemical tests which include; coagulase, indole, motility, citrate and urease as described by Bergeys manual of determinative bacteriology.

Identification of Microorganisms

The total bacteria count was calculated for the colonies examined on the media and sub culturing will be done to identify isolates. Different morphological features of the yielded colonies including color, size, elevation and pigmentation were recorded.

Gram Staining: A smear of colonies isolated was made on a glass slide using a wire loop. It was dried and heat-fixed. Then, the fixed smear was flooded with crystal violet solution for 60 seconds and washed. The fixed smear was then covered with Lugos iodine for 60 seconds. This was washed off and decolorized with ethanol 70% (note: ethanol is washed immediately to avoid over de-colorization). The smear was then flooded with Safranin solution for 60 seconds and then rinsed with water and air-dried and viewed under the microscope.

Biochemical Test

- **Indole Test**

Colonies was picked and inoculated into the test tube containing the indole medium and finally incubated at 37°C for 48 hours. Sometimes 96 hours at 37°C may be required. 0.5ml of kovacs reagents was added drop wise to the test tubes and shake gently. The production of indole is confirmed by the formation of red ring colorations on the surface of the medium, which indicates a positive reaction while; in negative reaction red colorations is not produced.

- **Oxidase Test**

This test identifies the presence of cytochrome c oxidase in microorganisms. It helps differentiate between oxidase-positive and oxidase-negative bacteria. Here a small colony of the bacteria was placed on a test strip or a slide. The compound tetramethyl-p-phenylene-diamine was added to the bacteria. The color of the bacteria was observed after a few minutes. If the bacteria change color to dark purple or blue, it means they have oxidase. If the bacteria remain the same color, it means they don't have oxidase.

- **Motility Test**

The motility test is aimed at identifying motile bacteria. A drop of normal saline was placed on a sterile slide and a colony of test organism was suspended and emulsified and then covered with a coverslip. The prepared slides were examined microscopically using 10x and 40x objective lens. Movement in different directions gave a positive test.

- **Urease Test**

Urease test is applied for bacteria species that can decompose urea by an enzymatic reaction to produce ammonia. After solidification of the urea medium, the inoculums was inoculated into the slant bottles and incubated at 37°C for 24 hours. A positive test is indicated by purple-pink color and for a negative test there is no change.

- **Citrate Test**

Kosers citrates medium was inoculated with the isolate and incubated at 37°C for 48hours. It was examined after two days. The presence of growth will lead to an increase in pH resulting to change in color for a positive test and initial green color for a negative test.

Soil Physicochemical Analysis

Soil Texture: This was carried out to determine the size of the soil particles. In carrying out this test, the Bouyoucos-

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type hydrometer method was used (Day P.R.O).

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

Water Holding Capacity: Determination of the water holding capacity of the soil samples was analyzed using the method called gravimetric method. In this method, soil samples were collected and dried in an oven to remove all the water. The weight of the dried soil sample was then measured. The sample was re-wetted to its field capacity, and the weight of the re-wetted soil was measured. The difference between the weights gives the water holding capacity of the soil [12].

Statistical Analysis

All data generated from the study were subjected to analysis of variance (ANOVA) using SPSS while means that were significantly different were further grouped using the Duncan multiple range test.

Results

This study was carried out to assess the effect of palm oil processing waste on soil Microorganisms. Table 4.1 shows the physicochemical parameters of both the control soil and three samples from three locations. It shows that the pH of the soil decrease compared to the control soil. The moisture content or water holding capacity of the soil samples increased compared to the control while there was a rise in temperature of the soil samples compared to the control soil. Table 4.2 shows that there was a decrease in the total colony count and total viable count of microorganisms in the sample soil compared to the control soil. There was also a decrease in the total Fungi count of sample soil compared to the control soil, though the decrease was not as high as that of the total bacteria count. Table 4.3 presents the Cultural, morphological and Biochemical characteristics of Bacteria and identification of these organisms. Bacteria suspect to be present were *E.coli*, *Klebsiella* spp, *Proteus* spp, *Staphylococcus* spp, *Bacillus* spp, and *Shigella* spp. Presented on Table 4.4 is the Microscopic and macroscopic characteristics of fungi. This table presented both macroscopic and microscopic features of fungi and the probable fungi. The fungi suspected were *Mucor* spp, *Rhizopus* spp, and *Aspergillus* spp. Table 4.5 shows the percentage prevalence of bacteria isolated with *klebsiella* having the highest prevalence while Table 4.6 shows the prevalence of fungi with *Rhizopus* spp and *Aspergillus* spp being the most prevalent.

Samples	pH	Moisture (%)	Temperature (°C)	Particulate Nature (%) Clay	Sand	Silt
N. Bank	5.70 ±0.14^{b}	44.29 ±0.127^{b}	28.40 ±0.141^{b}	6.35 ±0.212^{b}	75.00 ±0.283^{c}	11.55 ±0.353^{c}
Wurukum	5.35 ±0.21^{b}	48.52 ±0.275^{a}	29.20 ±0.141^{a}	6.25 ±0.494^{c}	76.45 ±0.919^{b}	17.30 ±1.414^{b}
H/Level	4.95 ±0.95^{c}	47.30 ±0.000^{a}	29.30 ±0.282^{a}	6.95 ±0.212^{a}	74.90 ±0.424^{d}	17.89 ±1.011^{b}
Control	6.30 ±0.00^{a}	37.05 ±0.212^{c}	27.95 ±0.212^{c}	4.70 ±0.141^{d}	77.10 ±0.283^{a}	18.20 ±0.141^{a}
P-value	0	0.01	0	0.01	0.01	0

Table 4.1: Physicochemical parameters of soil samples.
Means on the same column with different superscript are statistically significant (P<0.05)
KEY: N. bank= Northbank; H/Level= High Level

Samples	TCC(Cfu/g)	TVC (Cfu/g)	TFC (Cfu/g)
N.Bank	122.66 ±7.023^{c}	33.33 ±2.516^{b}	29.33 ±1.527^{bc}
Wurukum	204.00 ±7.549^{b}	23.66 ±2.081^{c}	31.66 ±4.161^{b}
H/Level	275.66 ±5.859^{a}	10.66 ±1.527^{c}	25.66 ±2.081^{c}
Control	291.00 ±13.00^{a}	118.33 ±18.717^{a}	44.33 ±1.527^{a}
P-Value	0	0	0

Table 4.2: Total Colony Count, Total Viable Count and Total Fungi Count.
Means on the same column with different superscript are statistically significant (P<0.05)
KEY: N. bank= Northbank; H/Level= High Level

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Sample	Appearance	Grams Rnx	Morphology	Catalase	Indole	Citrate	H2s	Urease	Motility	Suspected Organism
A1	Pink	-	Rod	+	-	-	-	-	-	*Escherichia. coli*
A2	purple	+	Cocci	+	-	-	-	+	+	*Klebsiella* spp
A3	Purple	+	Rod	+	-	-	-	+	+	*Klebsiella* spp
A4	Pink	-	Rod	+	-	+	+	-	-	*Proteus* spp
B1	Purple	+	Cocci	+	-	+	+	-	+	*Staphylococcus* spp
B2	Pink	-	Rod	+	-	-	-	+	-	*Klebsiella* spp
B3	purple	+	Rod	+	-	-	-	+	+	*Bacillus* spp
B4	Pink	-	Rod	+	-	-	-	-	-	*Shigella* spp
C1	Pink	-	Rod	+	-	+	+	-	-	*Proteus* spp
C2	purple	+	Cocci	+	-	+	+	-	-	*Staphylococcus* spp
C3	Pink	-	Rod	+	-	-	-	+	-	*Klebsiella* spp
C4	purple	+	Cocci	+	-	-	+	+	+	*Staphylococcus* spp

Table 4.3: Cultural, Morphological and Biochemical Characteristics of Bacteria**.**

Macroscopic	Microscopic	Suspected Organism
White to dark gray surface.	non-septate	*mucor* spp
black and white pigment	septate	*Rhizopus* spp
wooly white with orange spots	non-septate	*Rhizopus* spp
wooly white turned black	Septate	*Aspergillus* spp
Cotten white irregular shape, greenish yellow	Septate	*Aspergillus* spp

Table 4.4: Microscopic and Macroscopic Characteristics of Fungi.

Bacteria Isolates	Occurrence	Percentage %
E. coli	1	8.33
Klebsiella spp	4	33.33
Proteus spp	2	16.67
Staphylococcus spp	3	25
Bacillus spp	1	8.33
Shigella spp	1	8.33
Total	12	99.99

Table 4.5: Percentage Prevalence of Bacteria Isolated.

Fungi Isolated	Occurrence	Percentage %
Mucor spp	1	20
Rhizopus spp	2	40
Aspergillus spp	2	40
Total	5	100

Table 4.6: Percentage Prevalence of Fungi Isolated.

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Discussion

In this study, it was observed that there was a decrease in pH of the polluted soil samples compared to the control soil sample (pH decrease from 6.30 ±0.00 to 5.33 ±0.434 mean of pH from the three sites). The decrease in pH is associated with decay of organic matter in soil Palm oil processing waste (POPW) which alters the pH. This is in agreement with the work of Ong HKA, et al. [13] who opined that one major effect of palm oil processing waste on soil is the decrease in soil pH. It was also observed that there was rise in temperature in soil samples polluted with POPW compared to the control soil sample (a rise in temperature from 27.95 ±0.212 in control sample to 28.96 ±0.188 in the mean of samples from the three sites). This agrees with the study of Chin MS, et al. [14] which concluded that microbial decomposition of organic compounds in Palm oil processing waste releases heat as a byproduct, causing a temperature rise in the soil. It was also observed that there was an increase in moisture content of soil polluted by palm oil processing waste might be as a result of much organic matter present in POPW. This is in agreement with the work of Ong HKA, et al. [13].

Soil samples impacted with palm oil processing waste (POPW) yielded low microbial population and diversity when compared to soil samples without POPW. This is in agreement with work of Oyeleke SB, et al. [15]. He observed that there was a decrease of bacteria isolates in soil impacted with POPW which could be due to high organic content, which can lead to oxygen depletion and create unfavorable conditions for many soil microorganisms. This may disrupt the soil microbial community and impact overall soil health. The total Fungi count also dropped, but the effect was not as much as that of bacteria (a decrease from 44.33 ±1.527 to 28.88 ±2.590 mean of the three samples). This may likely be due to the fact that soil fungi may exhibit ability to break down tough organic matter and have resilience or adaptation mechanisms that mitigate the negative effects of POPW, leading to a perceived lesser impact. This aligns with the findings of Smith JL, et al. [16] but contradicts the study by Yeoh BG, et al. [17], which asserts that POPW leads to an elevation in soil fungi abundance.

Six Bacteria and three fungi were isolated from three samples in three different locations. They include (Bacteria) *Klebsiella, Proteus, Staphylococcus, Bacillus, Shigella spp and E.coli.* (Fungi) *Mucor* spp*, Rhizopus* spp, and *Aspergillus* spp which is in agreement with the work carried by Ong HKA, et al. [13] who isolated similar organisms. *Klebsiella* spp (33.33%) prominence suggests a significant presence in the studied samples, possibly indicating its adaptability or prevalence in the environment under investigation. The coexistence of *Staphylococcus* spp (25%) and *Proteus* spp (16.67%) highlights microbial diversity within the sample. *Staphylococcus* spp, known for its versatility, may contribute to this diversity alongside *Proteus* spp, indicating varied microbial sources. The comparable percentages of *Bacillus* spp (8.33%), *Shigella* spp (8.33%), and *E. coli* (8.33%) suggest a balanced distribution among these isolates. This is agreement with the findings of Orji MU, et al. [18]. This equilibrium may signify a dynamic microbial community, influenced by factors such as environmental conditions or host interactions. *Staphylococcus* spp and *Klebsiella* spp, with higher prevalence, raise concerns about potential health risks, considering their pathogenic nature. This warrants further investigation into the specific strains present and their associated virulence factors.

Conclusion

The examination of palm oil processing waste impact on soil microbial communities revealed significant consequences of palm oil processing waste on soil microorganisms. It was observed that there were significant differences in the determined soil physicochemical parameters of polluted soils when compared to the control sample. There were reduction in microbial population and diversity in soil samples impacted with palm oil processing waste (POPW) yield when compared to soil samples without POPW. Inadequate management could pose a substantial threat, as the persistent use of palm oil processing waste may induce alterations that negatively impact soil micro biota and subsequently soil fertility. This could result in diminished agricultural yields when the effluent is improperly disposed of on farmlands.

Recommendation

Based on the results of the findings, the following recommendations were made;

1. Implement a long-term monitoring strategy to capture changes in soil microbial communities over time, providing a more comprehensive understanding of the sustained effects of palm oil processing waste (POPW).
2. Formulate clear policy recommendations based on the study findings, aiming to guide government initiatives in regulating and managing the disposal of POPW to minimize environmental and agricultural impacts.
3. Develop and implement public awareness campaigns to educate farmers, industries, and the general public about the potential hazards of improper POPW disposal and the importance of adopting responsible waste management practices.

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