



ASSESSMENT ON THE MICROBIAL LOAD AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF ISOLATES FROM SELECTED PALM OIL MILL EFFLUENT (POME) IN NIMO, ANAMBRA STATE.

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Abstract: The microbial load and antibiotic susceptibility patterns of isolates from selected Palm oil mill effluent in Nimo, Anambra state was assessed. Four (4) samples each of palm oil effluent were collected from some local oil palm processing sites from the study area. After serial dilution, One milliliter of 1:10⁵ and 1:10⁶ dilution factor were distributed into a prepared nutrient agar and sabouraud dextrose agar plates. After the inoculation, the plates were incubated for 24hours at 37°C and bacterial count were obtained, thus 2.62 × 10³ cfu/ml, 1.40 × 10³ cfu/ml, while 2.20x 10⁶cfu and 1.52× 10⁶ cfu/ml for fungal count for highest and lowest count respectively. Using standard colony, morphological and biochemical identification methods, the organisms were observed and recorded. A total of four bacteria isolates were identified; *Bacillus spp*, *Clostridium spp*, *Micrococcus spp* and *Staphylococcus spp* while *Candida spp* and *Mucor spp* were identified as the fungi. The antibiotic susceptibility patterns of the bacterial isolates were determined against the following antibiotic agents: erythromycin (10 µg), ciprofloxacin (20 µg), ampiclox (10 µg), rifampicin (20 µg), amoxil (20 µg), septrin (30 µg), ampicillin (30 µg), ceporex (10 µg) and levofloxacin (20 µg). The study revealed that palm oil effluent harbor a diverse number of microorganisms which could be pathogenic and could encourage the spread of infectious disease, hence the need for the treatment of the effluent before discharging them to the environment. Also the study gives a clear understanding on the knowledge of the patterns of the antibiotics activities against the isolates. Therefore, contributes to a better understanding of possible damage that could be made to aquatic and soil ecosystem by polluted oil mill effluents, thus paves a way for developing improved preservation strategies to enhance the quality and safety of aquatic and soil inhabitants.

Keywords: Microbial Load, Antibiotic Susceptibility Patterns, bacteria, fungal isolates, Palm Oil Mill Effluent.

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1. Introduction

The oil palm (*Elaeis guineensis*) is the world's most efficient yielding crop in-terms of productivity, efficiency and land utilization. It produces about 4 to 5 tonnes of oil per hectare per year [1]. Palm oil is one of the most traded edible vegetable oil in the world derived from fruit flesh (mesocarp) of the oil palm. Each fruit comprises of a hard kernel (seed) with a shell (endocarp) surrounded by a fleshy mesocarp (reddish pulp) [2]. About 50% of the global traded vegetable oil comes from palm oil, the palm oil industries are based agro-based enterprise in Nigeria especially in the southern part where palm oil trees are found both in the wild and plantations. At room temperature, palm oil is

semi- solid due to its saturation level of about 50% and also naturally reddish in colour due to its high content of beta-carotene. Palm oil contains monosaturated oleic acid 40%, polysaturated linoleic acid 10% (an essential fatty acid), Palmitic acid 45% (a fatty acid by our body) and Stearic acid 5% [3]. Palm oil and its products at prolonged temperatures have good resistance to heat and oxidation thus making it an ideal ingredient in frying oil blends. It is also rich naturally in chemical compounds for nutrition and health.

The processing of palm oil is carried out in mills where oil is extracted from the palm fruits. The global market for palm oil has experienced rapid growth in recent decades with current production



of palm oil estimated at over 81million metric tonnes [4]. Apart from Palm oil and Palm kernel, the processing of oil Palm also produces copious amounts of waste commonly referred to as Palm oil mill effluent (POME), which comprise of 50% of the liquid waste, discharged onto soil and aquatic environment in a raw state by local mill operators more often [5].

A wet process is the common method adopted for extracting palm oil. For each ton of crude palm oil (CPO) produced, 5 to 7.5 tons of water are required and more than 50% of this water ends up as palm oil mill effluent (POME) at the final stage [6]. Palm oil mill effluent is a brownish voluminous mixture of water, oil, fine suspended components generated mainly from sterilization, clarification and hydrocyclone (separator) processes in milling oil palm. The suspended components consist mainly of vegetative matters like organelles, cell walls, water insoluble carbohydrate ranging from hemicelluloses to simple sugars (glucose, reducing sugars and pectin), short fibres, free organic acids, lipids as well as the assembly of minor organic and mineral constituents and nitrogenous compounds (from proteins to amino acids) which can be converted into useful materials using microbial processes [7]. The raw or partially treated Palm oil mill effluent has extremely high content of degradable organic matter, which is due in part to the presence of unrecovered palm oil. Untreated POME contains high amount of fatty acids, proteins, carbohydrates and other plant materials, which has the tendency of altering environmental parameters particularly BOD, C/N ratio and COD level [8].

Palm oil mill effluent (POME) is characterized by high volume and contains substantial quantities of organic matter, nutrients, suspended solids and toxic elements that can impact the environment when improperly managed [9]. The release of POME into the environment especially when left untreated can result into severe ecological imbalances, implications for soil physicochemical parameters and the diversity and abundance of soil flora which are integral components of terrestrial ecosystems and detrimental effects on surrounding ecosystem [10].

One of the most significant effects of palm oil mill effluent (POME) on soil is its impact on microbial diversity. POME is rich in organic matter and nutrients such as nitrogen and phosphorus, which can lead to an initial increase in microbial biomass due to the availability of a carbon source [11]. However, long-term application or excessive POME can disrupt the balance of soil microbial communities. The dominant microbial species may shift towards those that are better adapted to high nutrient loads, leading to a reduction in microbial diversity [12]. For instance, excessive nutrients may favor fast-growing bacteria like *Pseudomonas spp* while reducing the presence of more slow-growing, nutrient-sensitive microbes [13]. POME can also affect soil enzyme activities, which are key indicators of microbial function. While some enzymes may show increased activity due to the influx of nutrients, others may be inhibited by changes in soil pH, oxygen availability, or the presence of toxic substances in POME [14]. For example, dehydrogenase activity, which is an indicator of overall microbial metabolism, may initially increase but later decline as the soil environment becomes more anaerobic. Similarly, enzymes involved in nutrient cycling, such as phosphatases and ureases, may be inhibited by changes in soil chemistry caused by POME application, particularly due to shifts in soil pH.

Studies have shown that both bacteria and fungi thrive in palm oil mill effluent enriched environments which can either benefit or degrade soil quality. Common bacterial species includes; *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* which are involved in nutrient cycling but can also contribute to soil acidification when in excess [15]. Additionally, fungi such as *Aspergillus niger*, *Penicillium spp* and *Trichoderma viride* are frequently isolated from Pome - affected soils playing a role in organic matter degradation but also posing risk of contamination and toxicity if their growth becomes unregulated [13]. Some fungal diseases of poultry include aspergillosis, candidiasis, dactylariosis, cryptococcosis, favus, rhodotorulic infections, torulopsis, mucormycosis, histoplasmosis, and cryptococcosis. Moreso, some bacterial diseases of poultry include peritonitis, polyserositis, colibacillosis, mycoplasmosis (chronic respiratory disease), and salmonellosis [16]. Bacteroidetes: This bacterial group is involved in the degradation of polysaccharides and proteins. Bacteroidetes are abundant in environments with high organic content, such as POME, and play a significant role in breaking down plant materials [17]. These bacteria help convert complex organic matter into simpler molecules that can be utilized by other microorganisms in the microbial food web.

Actinobacteria, are present in lower numbers, while *Micrococcus luteus* and *clostridium sporogenes* have been reported to have contributed to the degradation of recalcitrant organic compounds, such as lignin and cellulose. These bacteria are known for their ability to produce enzymes that break down complex plant materials and are essential in the early stages of organic matter decomposition [18].

Antibiotic susceptibility testing (AST) is a laboratory procedure used to identify which antibiotic regimen is effective in treating specific patients. The ability of microorganisms to be killed or inhibited by a particular antibiotic is referred to as antibiotic susceptibility. Antibiotic susceptibility is crucial in selecting the appropriate antibiotic for treating specific bacterial and fungal infections [14].

Hence, understanding the microbial content of POME is crucial for evaluating the potential risks associated with palm oil industry waste disposal into the environment and to explore diverse developing sustainable mitigation strategies.

2. Materials and Method

Area of Study

The research was conducted in Nimo, Njikoka Local Government Area, Anambra State. Nimo is made up of four villages namely, Ifitenu, ifiteani, Etiti Nimo and Egbengwu and has Abagana and Enugwu-ukwu towns as its boundary towns. Majorly, Nimo people are farmers with special interest in palm oil mill business. Samples were collected from four (4) functional palm oil mill in the area, one from each village. .

Sample Collection

Four (4) samples labeled W (ifite enu mill), X (Ifite ani mill), Y (Etiti) and Z (Egbengu) of each Palm oil mill effluent were systematic collected randomly from four (4) very functional oil palm mill in each of the village in the study area using sterile sample bottles and they were immediately brought into microbiology laboratory Nnamdi Azikiwe University, Awka for

microbial analysis. The antibiotics used in this study were procured from a pharmacy store located within the study area. A total of nine (9) antibiotics were employed for susceptibility testing against the bacterial and fungal isolates. They includes: erythromycin (10 µg), ciprofloxacin (20 µg), ampiclox (10 µg), rifampicin (20 µg), amoxil (20 µg), septrin (30 µg), ampicillin (30 µg), ceporex (10 µg), levofloxacin (20 µg).

Isolation, characterization and identification of bacterial and fungal isolates.

The Enumeration and isolation of microorganisms was done using a previously described methods by [18]. One milliliter of the 10^{-5} and 10^{-6} dilution factor of the POME sample was distributed into prepared nutrient agar. The plates were incubated at 37°C for 24 hours and was examined thereafter. The colonies of organisms observed were sub-cultured on freshly prepared agar plates using sterilized inoculating loops into their appropriate media. Nutrient agar and Sabouraud Dextrose Agar (SDA) for bacteria and fungi respectively.

The biochemical tests employed in this study includes: Gram staining, motility, catalase, coagulase, oxidase, urease, voges-Proskauer, indole, methyl red, citrate utilization, sugar fermentation as well as Lactophenol Cotton Blue staining tests were carried out to identify the isolated organisms using previous standard methods as described by [16].

Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted on all bacterial isolates using the disc diffusion method. Pure bacterial isolates were evenly streaked individually on appropriately labeled agar plates. The inoculum from the bacterial pure cultures obtained from oil palm effluents was prepared by suspending bacterial cells in a sterile broth solution. Thereafter, the turbidity of the suspension was adjusted to match the McFarland standard. Subsequently, agar plates were inoculated with the broth culture containing the bacterial suspension using a sterile wire loop. Furthermore, the inoculums were allowed to dry at room temperature for 30 minutes. After drying, antibiotic-impregnated discs were carefully placed on the surface of the inoculated plates using sterile forceps. These antibiotic disks were carefully placed onto the agar surface and incubated. After 24 hours of incubation, the plates were then observed for bacterial growth, and zone of inhibition around each disk and was measured using a transparent meter rule. The result of susceptibility of each bacterial strain were interpreted by comparing the zone diameters of inhibition of each

drugs with the standards provided by the Clinical and Laboratory Standards Institute.

3. Results and Discussion

3.1. Plate count of bacterial isolates from Oil palm effluents samples

Table 1 shows the viable count of bacteria isolated from the selected palm oil mill effluents samples. It was revealed that Sample Y had the highest viable count of 2.62×10^5 cfu/ml, followed by Sample X with a viable count of 2.01×10^5 cfu/ml, while Samples Z and W recorded the least viable counts of 1.71×10^5 cfu/ml and 1.40×10^5 cfu/ml respectively.

Table 1: Total bacteria count of palm oil effluents samples

Palm Oil mill Effluents	10^5 cfu/ml
W	1.40
X	2.01
Y	2.62
Z	1.71

3.2 Plate count of fungal isolates from palm oil mill effluents samples

Table 2 revealed the viable count of fungal isolated from the selected oil palm mill effluents samples. It was revealed that Sample B had the highest viable count of 2.20×10^6 cfu/ml, followed by Sample A with a viable count of 1.95×10^6 cfu/ml, while Samples C and D recorded the least viable counts of 1.52×10^6 cfu/ml and 1.75×10^6 cfu/ml respectively.

Palm Oil mill Effluents	10^6 cfu/ml
A	1.40
B	2.01
C	2.62
D	1.71

3.3 Morphological and biochemical properties of bacteria from palm oil mill effluents samples

Table 3 shows the morphological characteristics of the bacteria isolates from POME. The morphological features were identified based on their shape, colour, elevation, margin and opacity on the plates.

Table 3: Morphological characteristics of the bacteria isolates from POME

POME Isolates	Shape	Colour	Elevation	Margin	Opacity
W	Circular	Cream	Convex	Entire	Opaque
X	Circular	Light-yellow	Convex	Entire	Opaque
Y	Circular	Cream	Undulate	Raised	Opaque
X	Irregular	White	Undulate	Flat	Opaque
O	Circular	Milky	Convex	Entire	Opaque

The biochemical properties of the above bacterial isolates are presented in Table 4. The table revealed the probable organisms that were identified thus; *Microoccus spp*, *Staphylococcus spp*, *Clostridium spp*, *Bacillus spp* and *Esherichia coli*.

Table 4: Biochemical properties of the bacteria isolates from POME

Isolates	Gram rxn	Form	OR	Cat	Ox	Ferm	Ur	Coa	Mot	SP	Probable organism
W	+	Cocco	A	+	+	-	+	-	-	-	<i>Mircococcus spp</i>
X	+	Cocci	FA	+	-	+	+	+	-	-	<i>Staphlococcus spp</i>
Y	+	Rod	AN	-	-	+	-	-	+	+	<i>Clostridium spp</i>
Z	+	Rod	FA	+	-	+	-	-	+	+	<i>Bacillus spp</i>
O	-	Rod	A	+	-	+	-	-	+	+	<i>Escherichia spp</i>

Key: rxn= reaction, + = positive, - = Negative, OR= oxygen requirement, Ca =Catalase test, Ox=oxidase test, Ferm= fermentation, A=Aerobe, FA= Facultative Anaerobe, AN=Anaerobe, Ur= Urease, Coa= Coagulate test, M=Motility, SP= Sporeformation.

3.4 Macroscopic and microscopic identification of fungal isolates from POME

Fungi isolated were grouped based on the macroscopic and microscopic identification as presented in table 5 below, which specify the type, colour, underside colour, texture, hyphae and spore formation. The identified fungal isolates are *Mucor spp* and *Candida spp*

Table 5: Macroscopic and microscopic characteristics of the fungal isolates from POME

POME Isolates	Fungal type	Colour	Underside colour	Texture	Hyphae	Sporeformer	Probable
W	Mould	White	Black	Woolly	Asptate	Sporangiosphore	<i>Mucor spp</i>
X	Yeast	Cream	Cream	Smooth	Septate	Conidiosphore	<i>Candida spp</i>
Y	Mould	White	Black	Woolly	Aseptate	Sporangiosphore	<i>Mucor spp</i>
X	Mould	White	Black	Woolly	Aseptate	Sporangiosphore	<i>Mucor spp</i>

3.5 Antibiotic susceptibility activities of the bacteria isolates from POME

Table 6 and 7 shows the antibiotic susceptibility test result for both gram negative and gram positive in millimeter respectively. In table 6 revealed that *Escherichia coli* was inhibited at a higher diameter of 22mm by ceporex and a least zone of inhibition of 6mm by levofloxacin. While Table 7 showed that *Clostridium spp* was the highest isolate to be inhibited by ceporex with zone of inhibition diameter of 23mm, while *Bacillus spp* recorded the least zone of inhibition diameter of 9mm, inhibited by ampiclox.

Table 6: Antibiotic susceptibility test result for gram-negative bacteria (mm)

Organism	ERN	CPX	AP X	RPN	AM X	SX T	AMP ,	CPX	LEV
<i>Escherichia coli</i>	-	14	-	10	12	-	15	22	6

Table 7: Antibiotic susceptibility test result for gram-positive bacteria (mm)

Organism	ERN	CPX	AP X	RPN	AM X	SX T	AMP ,	CPX	LEV
<i>Mircococcus spp</i>	10	-	12	-	12	-	-	-	-
<i>Staphlococcus spp</i>	-	17	10	-	10	-	10	23	11
<i>Clostridium spp</i>	15	19	10	-	-	-	19	-	-
<i>Bacillus spp</i>	-	10	9	-	-	-	10	-	-

Key: - = Resistace; mm=milliliter; ERN= Erythromycin (10µg); CPX=Ciproflaxacin (20µg); APX=ampiclox (10µg);

RPN=Rifampicin (20µg); AMX=Amoxil (20µg); SXT=Septrin(30µg);, AMP=Ampicilin (30µg) ,CPX=Ceporex (10µg), LEV=Levoflxacin (20µg),

It was revealed that Sample Y had the highest viable count of 2.62×10^5 cfu/ml, followed by Sample X with a viable count of 2.01×10^5 cfu/ml, while Samples Z and W recorded the least viable counts of 1.71×10^5 cfu/ml and 1.40×10^5 cfu/ml respectively. The findings of this study is in line with the study done by [19]. in their study on the Microbial load and biodegradation of POME by microorganisms at different stages of discharge, where the aerobic count of samples ranged from 2.1×10^6 cfu/ml – 1.9×10^6 cfu/ml. This implies that, high microbial load in POME is typical due to the organic richness of the effluent, which provides an ideal environment for microbial growth. The morphological features of the isolates were identified based on their shape, colour, elevation, margin and opacity on the plates. Thus, the probable organisms that were identified were *Microoccus spp*, *Staphylococcus spp*, *Clostridium spp*, *Bacillus spp* and *Escherichia coli*. This result agrees with the work done by [15], where *Micrococcus luteus* and *clostridium sporogenes* have been reported in POME, where they contribute to the degradation of recalcitrant organic compounds, such as lignin and cellulose. These bacteria are known for their ability to produce enzymes that break down complex plant materials and are essential in the early stages of organic matter decomposition. Similarly, Studies have shown that both bacteria and fungi thrive in palm oil mill effluent enriched environments which can either benefit or degrade soil quality. Common bacterial species includes; *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* which are involved in nutrient cycling but can also contribute to soil acidification when in excess as reported by [7]. Additionally, fungi such as *Aspergillus niger*, *Penicillium spp* and *Trichoderma viride* are frequently isolated from Pome were they play a major role in organic matter degradation but also posing risk of contamination and toxicity if their growth becomes unregulated as reported by [1]. The result of this study implies that microbial diversity promotes the growth of specialized microbes capable of surviving in polluted environments. These organisms may possess unique metabolic pathways or genetic traits that allow them to thrive under the stressful conditions created by effluent exposure [20]. Microbes with complementary functions can form consortia, working together to degrade pollutants more efficiently than individual species could. This synergistic interaction improves the capacity of the ecosystem to recover from pollution events, thereby supporting long-term ecological stability as indicated in the study.

The antibiotic susceptibility result of this study showed that *Escherichia coli* was inhibited at a higher diameter of 22mm by cephalexin, having the least zone of inhibition of 6mm by levofloxacin. It was also identified that *Clostridium spp* was the highest isolate to be inhibited by cephalexin with zone of inhibition diameter of 23mm, while *Bacillus spp* recorded the least zone of inhibition diameter of 9mm, inhibited by ampiclox. This shows that the activities of the bacteria in the POME could lead to the emergence of antimicrobial-resistance. The selective pressure exerted by the effluent, especially if it contains traces of agrochemicals or other pollutants, can encourage the proliferation of microbes with resistance to antibiotics or other antimicrobial agents. [21] supported the finding as written in his work on the effect of horizontal gene transfer between bacteria in effluent-impacted environments; through which resistance genes can be spread, posing a threat to public health. He further stated that, If pathogenic bacteria acquire resistance genes from environmental microbes exposed to POME, this can make infections harder to

treat in humans and animals, exacerbating the global issue of antimicrobial resistance.

4. Conclusion

The research showed a significant presence of some bacteria and fungi in palm oil mill effluent which has proven to pose a potential health risks and demands a need for stringent safety measures. The study also revealed that Erythromycin, ciproflaxacin, ampiclox, and amoxil were recommended for use as antibiotics to treat diseases that may be caused by some of these pathogens. This study underscores the importance of monitoring and controlling microbial contamination in palm oil mill effluent and its disposal to ensure safety and soil quality.

Competing Interest

The authors hereby declare that no competing interests exist regarding the publication of this research.

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