Applied Sciences Research Periodicals
October 2023, Vol. 1, No. 7, pp. 35-43

Impact of Palm Oil Mill Effluent on Soil Microflora Dynamics in Bekwarra L.G.A, Cross River State, Nigeria

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Abstract:
Palm oil production generally requires large input of water which is eventually discharged indiscriminately untreated, posing threat to soil biota. This research aimed to investigate the impact of Palm Oil Mill Effluent (POME) on soil microflora dynamics in Bekwarra L.G.A, Cross Rivers State Nigeria. Eight control sites were initially identified, and soil samples were collected from each site before any intervention. Subsequently, effluent was dumped on the sites, and soil samples were collected at regular intervals of four weeks for the duration of sixteen weeks. The collected samples were analysed to assess changes in soil biota. A total of 32 samples were collected at different stages of sampling period. Data obtained were analysed statistically using the SPSS (20.0 version). At week 16, bacteria populations reduced drastically where Afrike had the highest (1.5 × 108 cfu/g) while Abuochiche recorded the lowest (0.6 × 108 cfu/g). A total of 93 bacteria isolates were identified where Pseudomonas was the most dominant (38.7%) across the study site per and it was followed by Micrococcus (19.4%). In contrast, fungi count increased significantly at week 16 as the quantities of effluent on sites were high. Ukpah recorded the highest fungal count (4.8 × 105 cfu/g while the least (2.9 × 105 cfu/g) was recorded at Ibiaragidi. A total of 83 fungal isolates were recorded where Aspergillus was the most dominant (26.5%), followed by yeast (24.1%). The application of POME to the soil had a noticeable impact on the soil microbial community dynamics. The changes observed in the microbial population highlight the response of different microbial groups to POME, with bacteria exhibiting an early response while fungi showing a delayed but substantial increase. Further research is warranted to explore the specific mechanisms underlying these observed changes and their effects on soil health and ecological sustainability.

Keywords: Palm oil, Effluents, soil microflora, Impact assessment

INTRODUCTION

Palm Oil Mill Effluent (POME) is a by-product generated during the processing of palm fruits in palm oil mills. It is characterized by high volumes and contains a complex mixture of organic and inorganic substances, including oil, suspended solids, heavy metals, phenolic compounds, and nutrients (Ariffin *et al.*, 2013; Chong *et al.*, 2015). The release of POME into the environment, especially when left untreated, can result in 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 ecosystems (Ho, 2020).Therefore, understanding the effects of POME on soil properties and soil flora is crucial for evaluating the potential risks associated with palm oil industry waste disposal and developing sustainable mitigation strategies. POME, being rich in organic matter and nutrients, can influence microbial activity thereby affecting nutrient availability and microbial diversity in the soil ecosystem.

Soil flora, including bacteria, fungi, and plant species, contribute significantly to nutrient cycling, decomposition processes, and the overall stability of the ecosystem. The introduction of POME into the soil can disrupt the delicate balance of soil flora, leading to shifts in microbial community composition, decreased microbial diversity, and the proliferation of certain species adapted to the presence of POME (Ariffin *et al*., 2013). The microflora of palm oil mill effluent (POME) soil plays a vital role in the overall ecosystem of palm oil plantations and the management of POME waste because of their ability to resist, grow and utilizing POME (Nwaedozie *et al*., 2019). Microfloras commonly found in POME soil includes bacteria, fungi, Actinomycetes, Archaea and Algae (Yeoh *et al.,* 2016).

These alterations in soil flora dynamics can impact nutrient availability, carbon sequestration, and plant growth, thereby influencing the overall sustainability and productivity of agricultural systems (Jeremiah *et al*., 2014). To date, various studies have examined the effects of POME on soil flora. However, there is still a need for a comprehensive analysis that consolidates existing knowledge and identifies knowledge gaps to guide future research efforts. This research aims to address this gap by systematically investigating the impact of POME on a diversity, abundance, and functional attributes of soil flora in POME-affected areas. The general aim of the study was to assess the impact of palm oil mill effluent on soil microflora in Bekwarra palm oil production site, Cross River State, Nigeria.

MATERIALS AND METHODS

The Study Area

The study was conducted in Bekwarra Local Government Area of Cross River State, Nigeria. It is located between latitude 6.69°N of the equator and longitude 89.25°E with an area of 306 square kilometers (118sqmi). It has a population of 105, 822 as at 2006 National Population Census conducted (NPC, 2006) The LGA has ten wards which include; Abuochiche, Nyanya, Otukpuru, Gakem, Ibiaragidi, Ugboro, Ukpah, Beten, Afrike one, and Afrike two. It shares boundary with Vandeikya (Benue state) to the north, Obudu to the east, Ogoja at the south and Yala to the west. The average temperature ranged between 15° C and 30° C and the annual rainfall was between 1300-3000mm. All abiotic (climatic and edaphic) factors are favourable to palm tree cultivation. The study location which falls within the tropical rainforest makes it the ecological basis for production of wide range of tropical agricultural crops. The economic activity of the people in the study area is primarily on agriculture and commerce and the principal occupation of the people is farming. The cash crops include oil palm, groundnut and maize while the food crop includes; yam, cassava and cocoyam among others. Palm oil production is a common practice in Bekwarra. Generally, production requires input of large quantity of water which is eventually discharged as waste effluent into the surrounding environment untreated.

Sampling Techniques

A tour of the sampling area through all the eight (8) wards of the LGA was first undertaken and noted the differences between the sites in terms of the vegetation, soil colour, odour, presence of soil constituent, and moisture content. Eight new sites were selected as control areas, representing baseline soil conditions unaffected by effluent dumping.

Pre-Effluent Sampling

Soil samples were collected from each control site prior to any intervention, following established sampling protocols. The samples were labeled and appropriately stored for subsequent analysis.

Effluent Dumping
Palm oil mill effluent was dumped onto the same site continuously for the duration sixteen (16) weeks.

Sampling at Regular Intervals
Soil sampling was conducted at four-week intervals, starting immediately after the initiation of effluent dumping. At each sampling point, soil samples 0-20cm deep was collected from the same locations within each site using soil auger, ensuring consistency and comparability ( ). A total of 32 soil samples were collected at different stages of sampling period and transferred to Nasarawa State University Agronomy Research Laboratory, Lafia. The soil samples were mixed homogenously to obtain a composite sample for the eight production sites. All the soil samples were air-dried, crushed to finer particles and sieved using a 2mm sieve and then stored in clean polythene bag for 48 hours to ensure that there is stability of sample without alteration in their biological properties (APHA, 2005). The prepared samples were stored in labeled polythene bags for subsequent laboratory analysis.

Microbial Analysis
Serial Dilution:
Serial dilution was carried out by weighing 10g of soil into 90ml of sterile saline water in volumetric flask and shake thoroughly to dislodge the microorganisms from the soil particles. From this initial dilution, a ten-fold serial dilution was prepared (APHA, 2005).

Enumeration of Total Heterotrophic Indigenous Palm Oil Utilizing Bacteria:
The count of total heterotrophic bacteria in the soil sample was determined by pour plating 1ml of 10^{-9}ml into nutrient agar. The medium was incorporated with antifungal agent (funginox) to prevent the growth of fungal contaminant, and incubated for 48hours after which bacteria colonies on each of plate was enumerated and reported as colony forming units (cfu/ml) of the sample. Colonies were purified by repeated sub-cultured aseptically on fresh nutrient agar and incubated for 48 hours to obtain discrete pure colonies. For proper identification, gram staining was done for all the isolates (APHA, 2005).

Enumeration of Total Heterotrophic Indigenous Palm Oil Utilizing Fungal:
The standard procedures for serial dilution aforementioned for bacterial isolation were followed for fungal isolation. Thereafter, it was poured plated in potato Dextrose Agar (PDA) and supplemented with antibacterial agent (penicillin) to inhibit the growth of bacterial. The culture plate was incubated at 28^{o}C for 3days (APHA, 2005). After incubation, viable numbers of colonies on each plate were enumerated and recorded as colony forming unit per millimeter (cfu/ml). The colonies were sub-cultured into new PDA plate and incubated for 3days to obtain pure culture. Pure colonies were picked and stained for proper identification (APHA, 2005).

Identification of Bacterial Isolates:
All plates were examined and suspected colonies were identified by standard biochemical methods, and gram staining was carried out as described by Cheesbrough (2006).

Identification of Fungal Isolates:
Microscopic observation was carried out by preparing wet mount of culture fungus and observed under microscope. Also, potassium hydroxide (KOH) staining technique was used to enhance visibility of their structures as described by Alexopoulos *et al*. (2004).

Data Analysis

Data collected were recorded using Microsoft Excel package (2010) and imported into the SPSS (Statistical Package for the Social Science, 20.0 version) for further analysis. Statistics tool deployed was descriptive statistics and paired t-test to compare variables from palm oil mill effluent soil with non-effluent soil. Inferences were drawn using paired sample t-test, and chi-square test. Confidence level used was 95% level (p = 0.05).

RESULTS AND DISCUSSION

Table 1 compares total heterotrophic bacteria count of effluent soil of the entire site according to week with the control. Bacteria population on control soils were low across the sites, Nyanya recorded 1.61×10^8 cfu/g which was the highest count in the control samples, while Ukpah had the least 1.3×10^8 cfu/g. However, at week 4 when the effluent started accumulating, the entire sites recorded the highest bacteria count across the site. Otukpuru recorded 5.0×10^8 cfu/g which was the highest across the entire site; Nyanya had 3.8×10^8 cfu/g which was the lowest in week 4. Statistically, bacteria count at week four (4) was significantly higher ($t = 3.52$, $p<0.05$) when compared with the control.

At week 8, bacteria count dropped across the site. Otukpuru 4.4×10^8 cfu/g was the highest whereas Nyanya 3.3×10^8 cfu/g was the lowest. Bacteria count at week eight (8) was significantly higher ($t = 3.43$, $p<0.05$) over the control. At week 12, bacteria further drop as Otukpuru 3.2×10^8 cfu/g was the highest count, Ibiaragidi 2.0×10^8 cfu/g was the least across the sites. However, there was no significant difference ($t = 1.52$, $p>0.05$) when effluent soil data at week12 was compared with the control. At week 16, when effluent was high on the sites, bacteria populations further drop drastically; Afrike had the highest 1.5×10^8 cfu/g while Abuochiche recorded 0.6×10^8 cfu/g which was the lowest across the sites. Statistically, the value of effluent data was lower ($t = 1.3$, $p>0.05$) in record from week 16 and the control.

Table 2 compares total heterotrophic fungi count of effluent soil of all the sites with the control. The highest fungi count at the control soil was recorded at ugboro which was 1.35×10^5 cfu/g while the least was at Nyanya 1.21×10^5 cfu/g. At week 4, when effluent was introduced, fungi count decreased across the sites as the highest recorded was 1.3×10^5 cfu/g at Beten while Ukpah had 0.5×10^5 cfu/g which was the least. Fungi count at week four (4) was insignificantly lower ($t = 1.52$, $p>0.05$) when compared with the control. As the effluent increases at week 8, fungi population also increases. Beten recorded 2.2×10^5 cfu/g which was the highest while Otukpuru had 1.2×10^5 cfu/g which was the least. However, statistics show that fungi count at week eight (8) was significantly lower ($t = 1.43$, $p>0.05$) in record from week eight (8) and control. As effluent continue to increase at week 12, fungi population also increased. The highest was recorded at Afrike 3.0×10^5 cfu/g while Ukpah had 2.3×10^5 cfu/g which was the least. Nevertheless, fungi count was lower statistically ($t = 1.52$, $p>0.05$) when both week12 and the control were compared. At week 16, fungi count increased immensely as the quantities of effluent on sites were high. Ukpah recorded the highest count 4.8×10^5 cfu/g, while the least 2.9×10^5 cfu/g was recorded at Ibiaragidi. Statistics reveal fungi count obtained at week sixteen (16) was significantly higher ($t = 3.45$, $p<0.05$) in record from week 16 and control.

The research findings indicate a significant impact of palm oil mill effluent (POME) on bacteria populations in the soil over a 16-week period. In the control soil, bacterial growth was relatively low throughout the sites. However, at fourth weeks, when POME was introduced to the soil, bacterial growth experienced an exponential increase. This sudden surge in bacterial population

suggests that POME provides favorable conditions for bacterial growth, possibly due to the presence of organic nutrients in the effluent (Nwaugo *et al*., 2008). This aligns with the report by Tan *et al.* (2013) that lipolytic and cellulolytic bacteria and fungi thrive well in palm oil mill effluent soil because it is rich in nutrient like lipid and cellulosic materials. Surprisingly, from eight week through sixteen weeks, bacterial growth started to decline, even though POME was still present in the soil. This decrease in bacterial population could be due to various factors, such as nutrient depletion, competition, the presence of inhibitory substances in the POME or the development of unfavorable conditions for bacteria growth over time (Okwute *et al.*, 2014). Ariffin *et al.* (2013) reported same that excess POME in the soil leads to nutrient depletion which may result to inhibition of microbial growth and subsequently death.

Overall, the results demonstrate a declining trend in the number of bacteria isolates over the course of the 16-week study. suggesting a dynamic microbial community in the palm oil mill effluent, with potential shifts in dominant species and overall population size over time (Nwaedozie *et al.*, 2019). The study also suggests that there are significant differences in the distribution of the bacteria isolates within the palm oil mill effluent. In other words, the variation observed among the different bacteria isolates is not likely due to random chance alone. Based on the percentages, *Pseudomonas* spp was found to be the most dominant bacteria isolate, representing 38.7% of the total population. This suggests that *Pseudomonas* spp has a substantial presence within the palm oil mill effluent and has the ability to consumed organic carbon of POME soil (Ibiene *et al*., 2011). Okwute *et al.* (2014) reported that *Pseudomonas* spp are naturally associated with the degradation of palm oil and palm oil bearing materials. He stated that *Pseudomonas* spp are known to be capable of utilizing hydrocarbons as carbon and energy sources producing biosurfactant when grown on carbon surface. The presence of *Micrococcus* and other bacteria in palm oil mill effluent soils shows the ability to degrade oil at low pH (Jeremiah *et al.*, 2014). The results indicate that the observed differences in the distribution of bacteria isolates are unlikely to be due to random variation. Specific factors or conditions in the palm oil mill effluent may be influencing the prevalence and abundance of different bacteria species Jeremiah *et al.* (2018).

Table 1: Total heterotrophic bacteria count of effluent soil across sites with non-effluent soil.

	SITE	CONTROL	WEEK 4	WEEK 8	WEEK 12	WEEK 16
1	IBIARAGIDI	1.50×10^8	4.0×10^8	3.6×10^8	2.0×10^8	0.9×10^8
2	UKPAH	1.32×10^8	4.2×10^8	3.8×10^8	2.2×10^8	0.8×10^8
3	NYANYA	1.61×10^8	3.8×10^8	3.3×10^8	2.8×10^8	1.1×10^8
4	BETEN	1.55×10^8	4.3×10^8	3.8×10^8	2.3×10^8	1.4×10^8
5	UGBORO	1.41×10^8	4.6×10^8	4.0×10^8	2.5×10^8	1.2×10^8
6	OTUKPURU	1.58×10^8	5.0×10^8	4.4×10^8	3.2×10^8	1.1×10^8
7	ABUOCHICHE	1.52×10^8	4.8×10^8	4.2×10^8	2.4×10^8	0.6×10^8
8	AFRIKE	1.60×10^8	4.7×10^8	3.8×10^8	2.6×10^8	1.5×10^8
	T-test:		3.52, $p<0.05$	3.43, $p<0.05$	1.52, $p>0.05$	1.3, $p>0.05$

Table 2: Total Heterotrophic Fungi Count of Effluent Soil of All the Sites with Control According to Weeks

	SITE	CONTROL	WEEK 4	WEEK 8	WEEK 12	WEEK 16
1	IBIARAGIDI	1.30×10^5	0.7×10^5	1.7×10^5	2.6×10^5	2.9×10^5
2	UKPAH	1.33×10^5	0.5×10^5	1.5×10^5	2.3×10^5	4.8×10^5

3	NYANYA	1.21×10^5	1.2×10^5	1.6×10^5	2.9×10^5	3.3×10^5
4	BETEN	1.28×10^5	1.3×10^5	2.2×10^5	2.5×10^5	3.9×10^5
5	UGBORO	1.35×10^5	0.8×10^5	1.3×10^5	2.7×10^5	3.9×10^5
6	OTUKPURU	1.31×10^5	1.0×10^5	1.2×10^5	2.8×10^5	4.4×10^5
7	ABUOCHICHE	1.25×10^5	1.1×10^5	1.8×10^5	2.7×10^5	4.6×10^5
8	AFRIKE	1.25×10^5	0.9×10^5	1.5×10^5	3.0×10^5	3.5×10^5
	T-test:		1.52, p>0.05	1.43, p>0.05	1.52, p>0.05	3.45, p<0.05

Table 3 presents bacteria isolates obtained from effluent soil samples over a period of 16 weeks. At week 4, the total bacteria isolates identified were thirty-eight (38) which were the highest across the weeks. The most abundant bacteria isolate was *Pseudomonas* spp with eighteen (18) isolates, followed by *Micrococcus* spp with nine (9) isolates. *Staphylococcus* spp were six(6), *Proteus* spp three (3) isolates, *Bacillus* spptwo (2) isolates, while *Serratia* spp and *Clostridium* spp were not found. At week 8, the total number of bacteria isolates decreased to twenty-seven (27). The most prevalent bacteria isolate remained *Pseudomonas* spp with eight (8) isolates, followed by *Serratia* spp with six (6) isolates. *Bacillus* spp were four (4), while *Staphylococcus* spp and *Micrococcus* spp were three (3) each. *Clostridium* spp were two (2) while *Proteus* spp was the least identified with one (1) isolate. At week 12, the total number of bacteria isolates decreased further to twenty-one (21). *Pseudomonas* spp remained the most dominant with seven (7) isolates, followed by *Micrococcus* spp, *Serratia* spp and *Staphylococcus* spp with four (4) isolates respectively. *Bacillus* spp and *Clostridium* spp identified were one (1) each. At week 16, there was a drastic reduction in the total number of bacteria isolates. Only seven (7) isolates were identified. *Pseudomonas* spp continued to be the most abundant with three (3) isolates, followed by *Micrococcus* spp and *Serratia* spp with two (2) isolates each. Statistics reveals that distribution were not the same ($\chi^2 = 30$, $p<0.05$) when isolates at different weeks were compared. A total of 93 bacteria isolates were identified. *Pseudomonas* spp were thirty-six (36) which were the highest bacteria isolates across the entire sites representing 38.7% per total number of each isolate. *Micrococcus* spp were eighteen 18(19.4%), *Staphylococcus* spp thirteen 13(13.98%), *Seratia* spp were twelve 12(12.9%), *Bacillus* spp were seven 7(7.53%), *Proteus* four 4(4.3%) and *Clostridium* spp 3(3.23%).

Table 4 presents fungi isolates obtained across effluent soil samples at different stages of sampling period. The results obtained demonstrate a progressive increase in the number of fungi isolates over time, indicating a dynamic microbial community in the palm oil mill effluent soil. The dominance of different fungi species varied throughout the study. At week 4, fungi isolates were few, a total of seven (7) isolates were identified. *Candida* spp and *Penicillium* spp were the most prevalent with two (2) each, *Mucor* spp, *Aspergillus* spp, and *Rhizospus* were one (1) each while yeast isolate was not found. At week 8, there was a notable increase in the total number of isolates to twelve (12), with *yeast* becoming the most abundant group with four (4) isolates, *Aspergillus* spp isolated were three (3), *Candida* spp were two(2), *Mucor* were two (2), *Rhizopus* spp was one (1), and there was no *Penicillium* at week 8.Week 12 witnessed a further increase in the number of fungi isolates to twenty-six (26), particularly *Aspergillus* spp, which emerged as the dominant species with ten (10) isolates, followed by *Yeast* withfive (5)isolates, *Candida* spp and *Penicillium* spp were four (4) each, *Rhizopus* spp were two (2), and *Micrococcus* spp was one (1).In the final week (week 16), the total number of fungi isolates reached its highest point with thirty-eight (38) isolates across the sites. *Yeast* became the most prevalent group with eleven (11) isolates, followed by *Aspergillus* spp eight (8), *Penicillium* spp were seven (7). *Candida* spp also maintained a relatively high presence with six (6) isolates, while *Rhizopus*spp4 and *Mucor* had fewer isolates.

Distribution of isolates across weeks are not the same (χ^2 = 25.6, p<0.05). The percentage distribution of fungi isolated per number of each isolate from effluent soil samples shows that a total of 83 isolates were identified, *Aspergillus* spp were 22 which was the highest isolates across the entire sites representing 26.5% per total number of isolates. *Yeast* was 20(24.1%), *Candida* spp recorded was 14(16.87%), *Penicillium* spp were 13(15.66%), *Rhizopus* spp were 8(9.6%) and *Mucor* were 6(7.23%) which was the least across the effluent soil

The control soil exhibited a low population of fungi throughout the study sites. At fourth week, when POME was introduced, fungal growth remained low, indicating that POME had no immediate stimulatory effect on fungi (Iyakndue *et al.,* 2017). Interestingly, at eight-week, fungal growth started to increase despite the continued presence of POME. The sustained presence of POME until twelve week suggests that it may have facilitated the growth of fungi. The highest count of fungi was observed at sixteen weeks, coinciding with the highest level of POME on the soil. This finding suggests that fungi might have adapted or utilized the components in POME as a nutrient source, resulting in increased growth (Iyakndue *et al*., 2017). This conforms to the report of Bala (2016) that some fungi have high adaptive capacity to POME and can act in utilization of hydrocarbon.The introduction of POME had contrasting effects on bacteria and fungi populations in the soil. Bacteria initially showed a rapid increase in growth, followed by a subsequent decline. Fungi, on the other hand, exhibited a delayed response to POME, with their growth steadily increasing over time. The differences in response can be attributed to variations in nutrient requirements, competition, and the ability of different microbial groups to utilize components present in POME. Ahmad *et al*. (2016) reported that if POME is applied in excessive amounts or without proper nutrient management, can cause nutrient imbalances in the soil which may lead to inhibition of microbial growth. The alteration of the microbial composition and dynamics in the soil, affects the overall ecosystem balance (Ibrahim and Ijah, 2017).

These results indicate a dynamic fungal community in palm oil mill effluent soil, with different species displaying variations in their abundance over time. The presence of yeast and various filamentous fungi, including *Aspergillus* spp, *Candida* spp*, Penicillium* spp*, Rhizopus* spp, and *Mucor,* suggests a diverse and evolving microbial ecosystem in this environment. *Aspergillus*. The high prevalence of *Aspergillus* spp suggests its adaptability to the POME soil environment (Jeremiah*et al.*, 2018). Ibiene *et al.* (2011) reported that *Aspergillius* spp produce cellulase, the enzymes capable of degrading cellulose in palm oil mill effluent soil. *Yeast* accounted for 24.10% of the fungal population. *Yeast* showed a significant presence, indicating its ecological importance and potential contributions to the microbial community dynamics in the POME soil. *Candida* spp represented 16.87% of the fungal population. The presence of *Candida* spp suggests its role in the decomposition or utilization of organic matter present in the POME soil. This conforms with the report by Bala (2016) that *candida* spp and some fungi act in utilization of hydrocarbon in palm oil mill effluent soil. *Penicillium* spp accounted for 15.66% of the fungal population. *Penicillium* spp are known for their ability to break down complex organic compounds, indicating their potential role in nutrient cycling in the POME soil. *Rhizopus* spp are often associated with decomposition processes and can contribute to organic matter breakdown in the POME soil. Jeremiah *et al.* (2018) reveals that *Rhizopus* is a natural flora of the soil, and it's associated with Palm oil mill effluent. Although *Mucor* had the lowest percentage, its presence indicates its ecological niche in the POME soil ecosystem. The diverse distribution of fungal species in the POME soil suggests the existence of a complex microbial community, capable of decomposing organic matter and participating in nutrient cycling processes (Badmus *et al.,* 2014; Chai *et al.*, 2016).

Table 3: Bacteria Isolates Obtained from Effluent Soil Samples at different Stages of Sampling Period.

Week	*Pseudomonas* spp	*Staph.* spp	*Bacillus* spp	*Seratia* spp	*Micrococcus* spp	*Proteus* spp	*Clostridium* spp	Total
4	18	6	2	0	9	3	0	38
8	8	3	4	6	3	1	2	27
12	7	4	1	4	4	0	1	21
16	3	0	0	2	2	0	0	7
Total	36	13	7	12	18	4	3	93

$\chi^2 = 30$, df=18, $p<0.05$, Distribution, differs

Table 4: Distribution of Fungi Isolates Obtained from Effluent Soil Samples at Different Stages of Sampling Period.

Week	*Aspergillus* spp	Candida spp	Mucor spp	*Penicillium* spp	*Rhizopus* spp	Yeast	Total
4	1	2	1	2	1	0	7
8	3	2	2	0	1	4	12
12	10	4	1	4	2	5	26
16	8	6	2	7	4	11	38
Total	22	14	6	13	8	20	83
	$\chi^2 = 25.6$,	df 15,	$p<0.05$				

CONCLUSION

The bacteria count in the effluent soil showed a substantial increase at week four, followed by a decrease from week eight to week 16, indicating temporal changes in the microbial community. Furthermore, the fungal population in the effluent soil was initially low at week four but showed a significant increase from week eight through week 16. This suggests a potential adaptation of the fungal community to the accumulated effluent and highlights the need for studying the composition and ecological roles of these fungi. Finally, this research contributes to the understanding of the effects of palm oil mill effluent on soil and microflora, highlighting the importance of responsible waste management practices and sustainable soil management in the palm oil industry.

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