



Changes in Microbial Population of Palm Oil Mill Effluent Polluted Soil Amended with Chicken Droppings and Cow Dung

L. O. Okwute^{1*} and U. J. J. Ijah²

¹Department of Biological Sciences, University of Abuja, P. M. B. 117, Gwagwalada-Abuja, Nigeria.

²Department of Microbiology, Federal University of Technology, P. M. B. 65, Minna-Niger State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

Received 26th December 2013
Accepted 3rd February 2014
Published 12th February 2014

ABSTRACT

Aim of Study: To assess changes in microbial population in palm oil mill effluent (POME) polluted soil amended with chicken droppings and cow dung.

Study Design: 32 plots measuring 4 m² were mapped out in a randomized complete block design of five main plots with three replicates. Data collected were subjected to ANOVA using SPSS.

Place and Duration of Study: Faculty of Agriculture, Kogi State University, Anyigba, Kogi State, Nigeria: July 2011 to November 2011.

Methodology: Plots were polluted with palm oil mill effluent and subsequently remedied using varying amounts of chicken droppings and cow dung (5 kg, 10 kg and 15 kg). Microbiological analysis was carried out using Nutrient agar and Sabouraud dextrose agar for the enumeration of total aerobic heterotrophic bacteria (TAHB) and fungi (moulds and yeasts) respectively.

Results: Significant difference ($P=0.05$) in TAHB counts after 1 month and 2 months in all treatments with the exception of unamended polluted and unpolluted control soils. The counts however, increased after 2 months in all treatments with the exception of unamended polluted soil. The overall data suggest that amendment of the POME polluted soil enhanced microbial growth, particularly after 2 months meaning that bioremediation of

*Corresponding author: Email: lolookwute@yahoo.com;

the polluted soil can be achieved with the organic wastes within a short time.

Conclusion: Chicken droppings (at 10 kg and 15 kg/4m² plot) and a combination of chicken droppings and cow dung (at 10 kg and 15 kg/4m² plot) have the ability to significantly increase microbial populations in palm oil mill effluent (POME) polluted soil thereby stimulating the bioremediation of the polluted soil.

Keywords: Palm oil mill effluent (POME); chicken droppings; cow dung.

1. INTRODUCTION

Palm oil processing is carried out in mills where oil is extracted from the palm fruits. Large quantities of water are used during the extraction of crude palm oil from the fresh fruits and about 50% of the water results in palm oil mill effluent (POME) [1]. It is estimated that for 1 tonne of crude palm oil produced, 5 - 7.5 metric tonnes of water will end up as POME [2]. POME is usually discharged into the environment in either a raw or treated state. Raw POME consisting of complex vegetative matter is thick, brownish, colloidal slurry of water, oil and solids including about 2% suspended solids originating mainly from cellulose fruit debris, that is, palm fruit mesocarp [3]. The raw or partially treated POME has an extremely high content of degradable organic matter, which is due in part to the presence of unrecovered palm oil [2]. This highly polluting wastewater can, therefore, cause pollution of waterways due to oxygen depletion and other related effects [2]. It has been reported that heavy application of POME to soil significantly ($P \leq 0.05$) reduced the total aerobic heterotrophic bacterial populations in the soil when compared to counts for non-POME soil samples [4,5]. The POME also reduced phosphate solubilizing, nitrifying and lipolytic bacterial counts [5] and ammonium oxidizers were isolated from non-POME soil samples but not from POME polluted soil samples [6]. Microbial degradation appears to be the most environmentally friendly method of removal of oil pollutants since other methods such as surfactant washing and incineration lead to introduction of more toxic compounds to the environment [7]. The use of organic wastes (chicken droppings and cow dung) as cheap alternatives to procedures such as biopiling, membrane technology and activated sludge reactors is therefore an easy option for local mill operators for the reclamation of arable land. This study aims to assess the changes in microbial population in palm oil mill effluent (POME) polluted soil amended with chicken droppings and cow dung. Microorganisms present in the soil and organic wastes were identified and their potential utilization of POME was determined.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Palm oil mill effluent (POME) was obtained from an established oil mill on the outskirts of Anyigba Town, Kogi State, Nigeria. The effluent which is normally contained in a plastic drum was mixed thoroughly before being transferred into clean plastic containers, tightly screwed and transported to the laboratory in an ice box. When not in use the POME was stored in a refrigerator at 4°C. The organic wastes used were chicken droppings and cow dung. The chicken droppings was collected fresh from a poultry house (deep litter) in Gwagwalada, Abuja, Nigeria while cow dung was collected fresh from Gwagwalada abattoir, Abuja-Nigeria in polythene bags and transported to the laboratory. The organic wastes were sun-dried for 48 hours before being ground and packed in clean polythene bags.

2.2 Study Site

Randomized complete block design, (RCBD) was adopted. The land which was situated in a demarcated and secured area in the Faculty of Agriculture, Kogi State University, Anyigba, Nigeria was flat, non-sloping and well drained. It was ploughed, harrowed and mapped out into 5 main plots (80 m², 80 m², 80 m², 20 m², 20 m²). Three plots (80 m² each) representing those for cow dung, chicken droppings and a combination of the two organic wastes were subdivided into 9 sub-plots, each measuring 2 m by 2 m (4 m²) and a space of free land of 1 m by 2 m on each side of each plot to create adequate gaps (alleys) between plots. The remaining two main plots with an area of 20 m² each were subdivided into 3 plots of 2 m by 2 m with a gap (alley) of 1 m by 2 m in between plots. The two plots served as control 1 (soil alone) and control 2 (soil + POME).

2.3 Application of POME (Pollution)

On each sub-plot of 4 m², 12 litres of palm oil mill effluent (POME) was applied evenly using a garden watering can. This was done on all plots except control 1 (soil alone) which was left undisturbed. After the POME application, an auger was used to collect soil from all plots into properly labeled, clean polythene bags and transported to the laboratory for analysis. Soil samples were also collected after one month and two months.

2.4 Bioremediation of Polluted Soil

Two weeks after pollution, application of organic wastes was carried out. Cow dung was applied to each subplot measuring 4 m² in the following order, 5 kg (3 subplots), 10 kg (3 subplots), 15 kg (3 subplots). This was done by spreading the dried organic wastes evenly on each subplot. The same treatment was given to another set of 9 subplots for chicken droppings in the same order. The remaining 3 subplots received a combination of the two organic wastes in varying proportions (5 kg, 10 kg and 15 kg). No organic waste was applied to two main plots which served as control 1 (soil alone) and control 2 (soil + POME). After application of the wastes, adequate mixing of the wastes with the polluted soil was undertaken. Tilling was repeated once in two weeks throughout the period of the field experiment (two months). Soil samples were collected immediately after the application of the organic wastes and at 1 month interval for a period of two months into properly labeled, clean polythene bags and transported to the laboratory for microbiological analysis.

2.5 Microbiological Analysis

Microorganisms in the soil samples were enumerated by inoculating 0.1 ml serially diluted samples onto nutrient agar (NA) and Sabouraud Dextrose agar (SDA) plates for the enumeration of aerobic heterotrophic bacteria and fungi respectively using the spread plate method. The inoculated NA plates were incubated at 30°C for 48 hours while the SDA plates were incubated at 25°C for 3-5 days. Observed colonies were counted and expressed as colony forming units per gram (cfu/g) of soil.

2.6 Characterization and Identification of Microbial Isolates

2.6.1 Bacterial Isolates

Bacterial isolates were characterized based on Gram reaction and biochemical tests. The biochemical tests included production of coagulase, catalase, indole, urease, motility test, citrate utilization test, starch hydrolysis, Methyl Red-Voges Proskauer (MR-VP), triple sugar iron test, utilization of sodium azide and various carbohydrates (glucose, lactose, maltose, fructose, mannitol, sucrose and arabinose). The isolates were identified to the species level by comparing their characteristics with those of known taxa, as described in [8].

2.6.2 Mould Isolates

Mould isolates were characterized based on microscopic and macroscopic appearances which comprised pigmentation, colour of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spore, presence of special structures such as foot cell, sporangiophore or conidiophores and the characteristic of the spore head. The identities of the isolates were determined using the scheme of [9].

2.6.3 Yeast Isolates

Yeast isolates were Gram stained and characterized based on colonial morphology, cell micromorphology, germ tube and blastospore formation, gelatin liquefaction, starch hydrolysis, growth at 37°C and on 50% glucose and fermentation of the following carbohydrates: galactose, glucose, sucrose, maltose and lactose. The identities of the isolates were determined using the scheme of [10].

2.7 Statistical Analysis

Data generated from the study were analyzed using the computer package SPSS (Version 19.0) [11] which employed the use of univariate analysis of variance (ANOVA) at $P = 0.05$ confidence limit.

3. RESULTS AND DISCUSSION

3.1 Bacterial Counts in Soil Samples after Two Months of Bioremediation

The counts were statistically significant ($P = 0.05$) for bacteria in CKD (10 kg and 15 kg) and CD + CKD (10 kg) after 2 months of bioremediation (Table 1). The least bacterial counts was observed in POME polluted soil at zero time of bioremediation but this gradually increased in all treatments as bioremediation proceeded to the second month. The organic wastes with the most significant microbial counts at $P = 0.05$ confidence level were Chicken droppings (10 kg and 15 kg), Cow dung + Chicken droppings (10 kg and 15 kg). It can be seen from the results that chicken droppings generally had an edge in stimulating the growth of microorganisms in the polluted soils. All microbial counts decreased at the time of POME application and gradually increased over the period of bioremediation. This agrees with the findings of [5]. The decrease in counts may be directly related to the acidic nature of raw POME [3]. The gradual increase in microbial counts after the application of the organic wastes indicated that the nutrients in the wastes, possibly nitrogen and phosphorus helped

the microorganisms to overcome the initial stress experienced as a result of the POME application.

Table 1. Bacterial Counts in Soil Samples after Two Months of Bioremediation

Treatment	Bacterial counts (cfu/g)		
	Time (Months)		
	0	1	2
A	$3.2 \times 10^5 + 0.002$	$6.0 \times 10^5 + 0.012$	$1.1 \times 10^6 + 0.001$
B	$4.5 \times 10^5 + 0.001$	$9.8 \times 10^5 + 0.012$	$1.4 \times 10^7 + 0.002$
C	$5.4 \times 10^6 + 0.008$	$1.5 \times 10^6 + 0.001$	$1.7 \times 10^7 + 0.014$
D	$1.1 \times 10^6 + 0.05$	$4.5 \times 10^6 + 0.02$	$6.5 \times 10^6 + 0.002$
E	$1.9 \times 10^6 + 0.003$	$7.5 \times 10^6 + 0.003$	$1.9 \times 10^7 + 0.01^*$
F	$1.8 \times 10^6 + 0.018$	$2.3 \times 10^7 + 0.01$	$2.6 \times 10^7 + 0.03^*$
G	$4.4 \times 10^6 + 0.01$	$1.0 \times 10^6 + 0.001$	$1.0 \times 10^7 + 0.01$
H	$5.5 \times 10^6 + 0.02$	$1.2 \times 10^7 + 0.02$	$1.8 \times 10^7 + 0.002^*$
I	$6.7 \times 10^6 + 0.01$	$1.4 \times 10^6 + 0.004$	$1.1 \times 10^7 + 0.02$
J	$2.0 \times 10^4 + 0.01$	$4.0 \times 10^5 + 0.001$	$1.1 \times 10^5 + 0.03$
K	$4.8 \times 10^6 + 0.15$	$5.0 \times 10^6 + 0.01$	$5.4 \times 10^6 + 0.01$

Values are means of three replicates \pm standard error, *---Significant at $P = 0.05$

A=Cow dung 5 kg, B=Cow dung 10 kg, C=Cow dung 15 kg, D=Chicken droppings 5 kg, E=Chicken droppings 10 kg, F=Chicken droppings 15 kg, G=Cow dung + Chicken droppings 5 kg, H=Cow dung + Chicken droppings 10 kg, I=Cow dung + Chicken droppings 15 kg, J= Polluted unamended soil, K= Unpolluted soil.

3.2 Mould Counts in Soil Samples after Two Months of Bioremediation

In Table 2, the mould counts were significant in CKD (15 kg) and CD + CKD (10 kg) respectively. Mould counts in chicken droppings have previously been reported to be higher than in cow dung which has been given as a reason for the better performance of chicken droppings as bioremediating agents [12]. However, it can be seen from the table that values were significant ($P=0.05$) after two months of bioremediation.

Table 2. Mould Counts in Soil Samples after Two Months of Bioremediation

Treatment	Mould counts (cfu/g)		
	Time (Months)		
	0	1	2
A	$2.0 \times 10^2 \pm 0.003$	$2.0 \times 10^3 \pm 0.001$	$3.5 \times 10^3 \pm 0.001$
B	$2.2 \times 10^2 \pm 0.001$	$2.2 \times 10^3 \pm 0.001$	$4.0 \times 10^3 \pm 0.001$
C	$1.8 \times 10^3 \pm 0.002$	$5.0 \times 10^3 \pm 0.002$	$5.9 \times 10^3 \pm 0.02$
D	$2.2 \times 10^3 \pm 0.003$	$1.5 \times 10^3 \pm 0.001$	$4.0 \times 10^3 \pm 0.001$
E	$2.5 \times 10^3 \pm 0.01$	$3.3 \times 10^3 \pm 0.002$	$4.6 \times 10^3 \pm 0.03$
F	$3.0 \times 10^3 \pm 0.001$	$4.0 \times 10^3 \pm 0.001$	$6.0 \times 10^3 \pm 0.01^*$
G	$3.5 \times 10^3 \pm 0.003$	$5.5 \times 10^3 \pm 0.03$	$5.0 \times 10^3 \pm 0.004$
H	$3.8 \times 10^3 \pm 0.001$	$5.0 \times 10^3 \pm 0.001$	$6.5 \times 10^3 \pm 0.01^*$
I	$4.3 \times 10^3 \pm 0.002$	$5.6 \times 10^3 \pm 0.002$	$5.0 \times 10^3 \pm 0.003$
J	$2.0 \times 10^2 \pm 0.001$	$1.1 \times 10^3 \pm 0.001$	$3.0 \times 10^3 \pm 0.03$
K	$4.0 \times 10^3 \pm 0.01$	$5.5 \times 10^2 \pm 0.001$	$6.0 \times 10^3 \pm 0.02$

Values are means of three replicates \pm standard error, *---Significant at $P = 0.05$

A=Cow dung 5 kg, B=Cow dung 10 kg, C=Cow dung 15 kg, D=Chicken droppings 5 kg, E=Chicken droppings 10 kg, F=Chicken droppings 15 kg, G=Cow dung + Chicken droppings 5 kg, H=Cow dung + Chicken droppings 10 kg, I=Cow dung + Chicken droppings 15 kg, J= Polluted unamended soil, K= Unpolluted soil.

3.3 Yeast Counts in Soil Samples after Two Months of Bioremediation

For the yeast counts, they were significant in CD (15 kg), CKD (15 kg) and CD + CKD (15 kg) (Table 3). However, no growth of yeasts was detected in CD (5 kg and 10 kg) and POME polluted soil at zero time of bioremediation. This indicates that the POME had a negative impact on the presence of yeasts at the initial time of POME application. There was however, a gradual increase in counts which showed a recovery of the yeasts from the impact of the POME over a period of two months.

Table 3. Yeast Counts in Soil Samples after Two Months Bioremediation

Treatment	Yeast counts (cfu/g)		
	Time (Months)		
	0	1	2
A	No detectable growth	$3.3 \times 10^2 \pm 0.015$	$4.2 \times 10^3 \pm 0.02$
B	No detectable growth	$5.0 \times 10^2 \pm 0.018$	$6.2 \times 10^3 \pm 0.013$
C	$3.0 \times 10^2 \pm 0.001$	$4.7 \times 10^2 \pm 0.01$	$7.9 \times 10^3 \pm 0.03^*$
D	$1.2 \times 10^2 \pm 0.02$	$2.8 \times 10^2 \pm 0.04$	$3.9 \times 10^3 \pm 0.01$
E	$3.4 \times 10^2 \pm 0.01$	$4.3 \times 10^2 \pm 0.002$	$5.3 \times 10^3 \pm 0.03$
F	$4.1 \times 10^2 \pm 0.01$	$7.0 \times 10^2 \pm 0.01$	$6.5 \times 10^3 \pm 0.02^*$
G	$3.5 \times 10^2 \pm 0.02$	$4.2 \times 10^2 \pm 0.02$	$5.4 \times 10^3 \pm 0.01$
H	$4.0 \times 10^2 \pm 0.01$	$5.2 \times 10^2 \pm 0.03$	$6.0 \times 10^3 \pm 0.02$
I	$5.3 \times 10^2 \pm 0.001$	$6.3 \times 10^2 \pm 0.01$	$7.5 \times 10^3 \pm 0.02^*$
J	No detectable growth	$1.3 \times 10^2 \pm 0.01$	$3.0 \times 10^3 \pm 0.01$
K	$1.5 \times 10^3 \pm 0.02$	$1.8 \times 10^3 \pm 0.02$	$1.9 \times 10^3 \pm 0.01$

Values are means of three replicates \pm standard error; *---Significant at $P = 0.05$

A=Cow dung 5 kg, B=Cow dung 10 kg, C=Cow dung 15 kg, D=Chicken droppings 5 kg, E=Chicken droppings 10 kg, F=Chicken droppings 15 kg, G=Cow dung + Chicken droppings 5 kg, H=Cow dung + Chicken droppings 10 kg, I=Cow dung + Chicken droppings 15 kg, J= Polluted unamended soil, K= Unpolluted soil.

3.4 Occurrence of Bacteria in Amended Palm Oil Mill Effluent (POME) Polluted Soil

In Table 4, *Bacillus subtilis* and *Pseudomonas aeruginosa* had the highest frequency of occurrence of 100% and 91% respectively after two months of bioremediation while *Staphylococcus aureus* occurred least frequently (39.4%) occurring in only two treatments (cow dung, 10 kg and unpolluted soil) at zero time of POME application. *E.coli*, *Proteus vulgaris* and *Micrococcus roseus* had frequencies of 94%, 91% and 76% respectively with *M. roseus* not occurring in POME polluted soil at all throughout the period of bioremediation (0-2 months). *Proteus vulgaris* was absent initially for most treatments but was detected at the end of the bioremediation process for almost all the soil treatments (Table 4). This indicates that it was greatly affected by the acidic nature of the raw POME on application [2]. The presence of *P.vulgaris* up to the second month of bioremediation corroborates the report of [13] in which the hydrocarbon biodegrading potential of *P. vulgaris* in oil polluted sites was reported.

Table 4. Occurrence of Bacteria in Amended Palm Oil Mill Effluent Polluted Soil

Treatment/ Time (months)	Bacterial Isolates																	
	<i>Pseudomonas aeruginosa</i>			<i>Bacillus sp.</i>			<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>			<i>Proteus vulgaris</i>			<i>Micrococcus roseus</i>		
	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
A	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
B	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
C	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+
D	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+
E	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
F	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
G	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+
H	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+
I	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+
J	-	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	-	-
K	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

A=Cow dung 5 kg, B=Cow dung 10 kg, C=Cow dung 15 kg, D=Chicken droppings 5 kg, E=Chicken droppings 10 kg, F=Chicken droppings 15 kg, G=Cow dung + Chicken droppings 5 kg, H=Cow dung + Chicken droppings 10 kg, I=Cow dung + Chicken droppings 15 kg, J= Polluted unamended soil, K= Unpolluted soil, + = Presence of bacteria, - = Absence of bacteria.

3.5 Occurrence of Moulds in Amended Palm Oil Mill Effluent (POME) Polluted Soil

Table 5 shows the dominance of *Aspergillus sp.* (94%) and *Penicillium verrucosum* (100%) over other fungi isolates. *Rhizopus oryzae* occurred least frequently (27.2%) with it occurring in only two treatments out of the eleven treatments at zero time and after one month of bioremediation. Other moulds such as *Mucor mucedo*, *Fusarium spp.*, *Trichophyton spp.* and *Trichoderma harzianum* had frequencies of 69.7%, 87.9%, 69.7% and 63.6% respectively. However, *Rhizopus oryzae* and *Trichoderma harzianum* were not detected in POME polluted soil throughout the period of bioremediation. The moulds isolated from the amended polluted soil in the field were genera of *Rhizopus*, *Aspergillus*, *Mucor*, *Fusarium*, *Trichophyton*, *Paecilomyces*, and *Penicillium* (Table 5). This means that these fungi are widespread in the soil [14]. The breakdown of petroleum hydrocarbons by moulds particularly of the genera *Aspergillus*, *Trichoderma*, *Penicillium*, *Mucor* and *Fusarium* has been reported by several authors [12,15,16]. *Aspergillus sp.* in particular are reported to be good producers of cellulases; the enzymes responsible for the breakdown of cellulose in POME [17,18]. Fungi are notably aerobic and can also grow under environmentally stressed conditions such as low pH and poor nutrient status [19,20].

3.6 Occurrence of Yeasts in Amended Palm Oil Mill Effluent (POME) Polluted Soil

In Table 6, the occurrence of yeasts was more frequent in *Candida albicans* (97%) and *Rhodotorula rubra* (81.8%) and least in *Saccharomyces cerevisiae* (45.5%). No growth was observed for *Saccharomyces cerevisiae* at all in two treatments (cow dung, 10 kg and cow dung/chicken droppings, 10kg). Also, *Torulopsis candida* with a frequency of 72.7% was not detected in cow dung/chicken droppings (10 kg) while *Rhodotorula rubra* was not observed at all in unpolluted soil throughout the period of observation. This indicates that it was introduced by POME and the organic wastes. *Rhodotorula* species have however been reported to be oil degraders [12] and in particular, good degraders of anthracene in soil [21]

The ability of the yeasts isolated (*Saccharomyces cerevisiae*, *Torulopsis candida*, *Rhodotorula rubra* and *Candida albicans*) to degrade POME which was demonstrated by their moderate frequency of occurrence (Table 6) in the field has also been reported by [12] though as petroleum utilizers.

Table 5. Occurrence of Moulds in Amended Palm Oil Mill Effluent Polluted Soil

Treatment /Time months	Mould Isolates																				
	<i>Aspergillus</i> sp.			<i>Mucor</i> <i>mucedo</i>			<i>Penicillium</i> <i>verrucosum</i>			<i>Fusarium</i> sp.			<i>Trichophyton</i> sp.			<i>Rhizopus</i> <i>oryzae</i>			<i>Trichoderma</i> <i>harzianum</i>		
	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2			
A	-	-	+	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+		
B	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+		
C	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+		
D	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+		
E	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+		
F	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+		
G	+	+	+	+	-	-	+	+	+	+	+	+	-	+	-	-	-	-	+		
H	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+		
I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+		
J	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-		
K	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-		

A=Cow dung 5 kg, B=Cow dung 10 kg, C=Cow dung 15 kg, D=Chicken droppings 5 kg, E=Chicken droppings 10 kg, F=Chicken droppings 15 kg, G=Cow dung + Chicken droppings 5 kg, H=Cow dung + Chicken droppings 10 kg, I=Cow dung + Chicken droppings 15 kg, J= Polluted unamended soil, K=Unpolluted soil, += Presence of moulds, -= Absence of moulds.

Table 6. Occurrence of Yeasts in Amended Palm Oil Mill Effluent Polluted Soil

Treatment /Time months	Yeast Isolates											
	<i>Candida albicans</i>			<i>Saccharomyces</i> <i>cerevisiae</i>			<i>Torulopsis</i> <i>candida</i>			<i>Rhodotorula</i> <i>rubra</i>		
	0	1	2	0	1	2	0	1	2	0	1	2
A	+	+	+	-	-	-	-	+	+	-	+	+
B	+	+	+	+	-	-	+	+	+	+	+	+
C	+	+	+	+	-	+	+	+	+	+	+	+
D	-	+	+	+	-	-	+	-	+	+	+	+
E	+	+	+	+	+	+	+	+	+	+	-	+
F	+	+	+	-	+	+	+	+	-	+	+	+
G	+	+	+	+	-	-	+	-	+	+	-	+
H	+	+	+	-	-	-	-	-	-	+	+	+
I	+	+	+	-	+	+	-	+	+	+	+	+
J	+	+	+	-	+	+	-	+	+	+	+	+
K	+	+	+	+	+	+	+	+	+	-	-	-

A=Cow dung 5 kg, B=Cow dung 10 kg, C=Cow dung 15 kg, D=Chicken droppings 5 kg, E=Chicken droppings 10 kg, F=Chicken droppings 15 kg, G=Cow dung + Chicken droppings 5 kg, H=Cow dung + Chicken droppings 10 kg, I=Cow dung + Chicken droppings 15 kg, J= Polluted unamended soil, K= Unpolluted soil, += Presence of yeasts, -= Absence of yeasts.

4. CONCLUSION

Organic wastes are known to have the ability to improve soil physical properties, buffer the soil and improve aggregate stability and the population of soil microorganisms. This indicates that a combination of the different microorganisms in the organic wastes and the conditions in the field provided conducive environment for growth and production of competent enzymes which helped in the breakdown of the organic compounds contained in the POME.

It is therefore concluded that chicken droppings (at 10 kg and 15 kg/4m² plot) and a combination of chicken droppings and cow dung (at 10 kg and 15 kg/4m² plot) have the ability to significantly increase microbial populations in palm oil mill effluent (POME) polluted soil thereby stimulating the bioremediation of the polluted soil.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the support of Mr. Ikechukwu Ogonnaya and Mr. Stephen Abu of the Soil Science departments of F.U.T. Minna and Kogi State University, Anyigba respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Poku K. *Small-Scale Palm Oil Processing in Africa*. Technology and Engineering, Food and Agriculture Organization of the United Nations, Rome, Italy; 2002.
2. Ahmad A, Ismail S, Bhatia S. Water recycling from palm oil mill effluent (POME) using membrane technology. *Desalination*. 2003;157:87-95.
3. Bek-Nielsen C, Singh G, Toh TS. Bioremediation of palm oil mill effluent. In: *Proceedings of the Porim International Palm Oil Congress 1999*; Istana Hotel, Kuala Lumpur, Malaysia; 1999.
4. Okwute OL, Isu NR. Impact analysis of palm oil mill effluent on the aerobic bacterial density and ammonium oxidizers in a dumpsite in Anyigba, Kogi State. *African Journal of Biotechnology*. 2007a;6(2):116-119.
5. Adebuseye SA, Ilori MO, Nwaugo VO, Chinyere GC, Inyang CU. Effects of palm oil mill effluent (POME) on soil bacterial flora and enzyme activities in Egbama. *Plant Products Research Journal*. 2008;12:10-13.
6. Okwute OL, Isu NR. The environmental impact of palm oil mill effluent (POME) on some physicochemical parameters and total aerobic bioload of soil at a dump site in Anyigba, Kogi State, Nigeria. *African Journal of Agricultural Research*. 2007;2(12):656-662.
7. Oboh BO, Ilori MO, Akinyemi JO, Adebuseye SA. Hydrocarbon Degrading Potentials of Bacteria Isolated from a Nigerian Bitumen (Tarsand) Deposit. *Nature and Science* 2006;4(3):51-57.
8. Buchanan RE, Gibbons NE. *Bergey's Manual of Determinative Bacteriology*. 8th ed., Williams and Wilkins Co., Baltimore; 1974.
9. Domsch KH, Gams W. *Fungi in Agricultural Soils*. 1st Edition, Longman Group Ltd., London, UK; 1970.
10. Barnett JA, Pankhurst, RJ. *A New Key to Yeasts*. North Holland Publishing Company. Amsterdam, Netherlands; 1974.
11. *Statistical Package for Social Sciences, SPSS*. Computer package for Windows, Version 19.0; 2010. Available: <http://www.spss.com>
12. Obire O, Anyanwu EC, Okigbo, RN. Saprophytic and crude oil-degrading fungi from cow dung and poultry droppings as bioremediating agents. *Journal of Agricultural Technology*. 2008;4(2):81-89.
13. Ceyhan N. Biodegradation of pyrene by a newly isolated *P. vulgaris*. *Scientific Research and Essays*. 2012;7(1):66-77.

14. Awad AHA. Vegetation: A source of air fungal bio-contaminant. 2005;21:53-61.
15. Ibiene AA, Orji FA, Ezidi CO, Ngwobia CL. Bioremediation of hydrocarbon contaminated soil in the Niger Delta using spent mushroom compost and other organic wastes. Nigeria Journal of Agriculture, Food and Environment. 2011;7(3):1-7.
16. Eze VC, Owunna ND, Avoaja DA. Microbiological and Physicochemical Characteristics of Soil receiving palm oil mill effluent in Umuahia, Abia State, Nigeria. Journal of Natural Science Research. 2013;3(7):163-169.
17. Wong KM, Nor AA, Suraini A, Vikineswary S, Mohd AH. Enzymatic hydrolysis of palm oil mill effluent solid using mixed cellulases from locally isolated fungi. Research Journal of Microbiology. 2008;3(6):474-481.
18. Mohanram S, Amat D, Choudhary J, Arora A, Nain L. Novel perspectives for evolving enzyme cocktails for lignocellulose hydrolysis in biorefineries. Sustainable Chemical Process. 2013;1:1-15. doi:10.1186/2043-7129-1-15
19. Davis JB, Westlake DWS. Crude oil utilization by fungi. Canadian Journal of Microbiology. 1979;25:146-156.
20. Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A. Bacterial-Fungal Interactions: Hyphens between Agricultural, Clinical, Environmental and Food Microbiologists. Microbiology and Molecular Biology Reviews. 2011;75(4):583-609. doi:10.1128/MMBR.00020-11.
21. Krivobok S, Miriouchkhine E, Seigle-Murandi F, Benoit-Guyod JL. Biodegradation of Anthracene by Soil Fungi. Chemosphere. 1998;37(3):523-530.

© 2014 Okwute et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history.php?iid=413&id=11&aid=3618>