Greener Journal of Microbiology and Antimicrobials
Vol. 6(1), pp. 1-7, 2021
ISSN: 2354-2284

https://gjournals.org/GJMA

Bioremediation Of Palm Oil Mill Effluent (POME) Contaminated Soil Using Cow Dung.

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ARTICLE INFO

Article No.: *082420107*
***Type*:** *Research*
Full text: *HTML, EPUB*

Accepted: *26/08/2020*
Published: *08/12/2021*

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Keywords: *Palm Oil Mill Effluent (POME), bioremediation, Cow dung, contaminated soil.*

ABSTRACT

Release of Palm Oil Mill Effluent (POME) into the environment can cause soil and freshwater pollution and can affect downstream biodiversity. Bioremediation of POME contaminated soil collected from palm oil processing mill at Omuahunwo community in Aluu, Ikwerre Local Government Area of Rivers State, Nigeria was carried out using cow dung as nutrient supplement. The monitoring period lasted for 14 days. Results of the physicochemical parameters showed reduction in values of Biological Oxygen Demand (BOD) from 160.10mg/l to 90.20mg/l, Chemical Oxygen Demand (COD) (120.40 - 70.2mg/l), Total Organic Content (TOC) (140.10 - 100mg/l) respectively. Low pH value of 4.11 before treatment, being acidic, was increased to 6.10 being less acidic. The total heterotrophic bacterial (THB) population increased from $3.0x10^6$ to 8.3×10^8 cfu/g and that of total fungi count increased from $3.2x10^4$ to 3.8×10^6 cfu/g respectively. Characterization and identification tests revealed that the bacterial genera encountered in the bioremediation process were; *Proteus, Bacillus, Escherichia coli, Pseudomonas, Micrococus and Corynebacterium* while the fungal genera were *Fusarium, Aspergillus, Pernicillium, Geothricum and Mucor.* Yeast was *Candida* sp. The results indicates that cow dung could be applied in the bioremediation of POME contaminated soil which is enriched with microorganisms with high degradative potentials in degrading the pollutants in POME contaminated soil for ecofriendly bioremediation technology.

INTRODUCTION

Palm oil processing is carried out in mills where the oil is extracted from the fleshy mesocarp of the fruit of oil palm (*Elaeis gunineensis*). Large quantity of water is being used during the extraction process, about 50 percent of water results in palm oil mill effluent (POME). It is estimated that for 1 ton of crude palm oil produced, 5-7.5 tons of water will end up as POME (Ahmad *et al.,* 2003).

It has been observed that most of the POME produced by small-scale traditional operators in Nigeria undergo little or no treatment and is usually discharged into the surrounding environment (Okwute, 2007).

POME is a brown slurry of organic solids (4-5%), residual oil (0.5-1.0%) and water (95%) which is generated mainly from palm oil extraction, washing and cleaning processes in the mill (Agamuthu, 1995). POME is characterized with high organic content, high

Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), (Maheswaran and Singam, 1977). It is known to cause environmental adverse effect such as eutrophication and freshwater pollution. The effect of release of untreated POME into the environment has been reported, leading to loss of biodiversity and soil fertility. Raw POME or partially treated POME usually contains extremely high content of degradable organic matter which is due to the presence of unrecovered palm oil (Ahmed *et al.,* 2003). It has also been reported that POME alters the physicochemical properties of soil, (Okwuta and Isu, 2007), pollution of water ways due to depletion of oxygen (Blek-Niel *et al.,* 1999). Significantly, it alters microbial populations in POME polluted soil (Nwaugo *et al.,* 2008) and reduction in the growth of oil palm seedlings (Nazeeb *et al.,* 1984).

Degradation of pollutants in the natural environment takes place slowly by activities of microorganisms. This will result to harmful effect in the ecosystem before such environment recovers. The roles of total aerobic bacteria and ammonium oxidizers are very crucial to the fertility of agricultural soil quality. It has been reported that the total aerobic bacteria help in the degradation of macromolecules from plants and animal remains, regulate most elemental cycles in nature and reduce the effects of pollutants in the soil by breaking them down to none toxic product that do not affect the environment (Dommergues, 1992, Khan, 2011, Nazeeh *et al.,* 1984).

In order to hasten the rate of recovery of polluted environments, bioremediation technologies are applied. Bioremediation is the use of biological processes and agent especially microorganisms their enzymes and green plants to degrade the environmental contaminants into less toxic forms, thereby returning the natural environment altered by pollutants to its original condition (Vidali, 2001; Khan, 2011). The rate of recovery will greatly depend on the nature of pollutant, the length of period and the bioremediation technique applied.

Some properties of POME which makes it imperative for bioremediation includes; long chain hydrocarbons and unrecovered oil, high organic load, toxicity effect due to the presence of phenols and other organic acids which are responsible, for phytotoxic effect and antibacterial activity. However, the polyphenolic fraction degrades with time and partially transforms to humid substances (Okwute and Isu, 2007).

Cow dung is the undigested residue of plant matter which has passed through the animals (cow) gut. It is a vast reservoir of nutrients and energy capable of supporting microbial growth, thereby enhancing microbial degradation of various polluted sites (Obire and Akinde, 2008). Cow dung is often used as manure (bio-fertilizer) for improving soil fertility for crop production and enhanced microbial activities which are important for natural biogeochemical processes (Obire and Akinde, 2008; Adebusoye *et al.,* 2007).

One of the major problems faced by palm oil producing industries in Nigeria, is the disposal of untreated palm oil mill effluent (POME) into large scale land used for farming. This problem demand that appropriate strategy be explored for the treatment (remediation) of POME contaminated soil. Such strategy should be efficient, cost effective and environmentally friendly. Hence the study on bioremediation of Palm Oil Mill Effluent contaminated soil in Aluu community of Rivers State using cow dung.

MATERIALS AND METHODS

Sample Collection

Palm Oil Mill Effluent (POME) polluted soil sample was collected from a polluted site around Palm Oil Mill located at Omuahunwo Aluu Community in Ikwerre Local Government Area of Rivers State, South-South, Nigeria. Bulked composite samples were collected using soil auger. The samples were pooled together for homogeneity into sterile black polyethylene bag and transported to the laboratory and stored in the refrigerator at 4^0C.

Cow dung was obtained from an abattoir in Aluu community, Ikwerre Local Government Area of Rivers State, Nigeria. The cow dung was collected into sterile polythene bag. It was composted for two weeks to reduce its pathogenic effect on the environment (Sample *et al.,* 2001).

Reagents and Media

All reagents employed in this study were of analytical grade and were products of Sigma Chemical Company, St. Louis, Missoun, USA and BDH Chemical, Ltd, Poole, England. All microbiological media used were products of Oxoid and Difco Laboratories England (Nurieut Agar (NA), Patator dextrose agar (PDA) and MacConkey's agar).

Experimental Setup

Two hundred and fifty grams (250g) of POME contamination soil was weighed and placed in two separate plastic containers. Set A contained the POME contaminated soil (PCS) while set B was added (amended) with 100g of dried and marshed cow dung (PCS + CD). Monitoring was done for 14 days (0,7 and day 14) respectively with microbiological and physicochemical analysis measured at intervals of 7 days. Each set up was mixed properly with wooden spatula to enhance aeration and optimum microbial activities and allowed to stand at room temperature (28 ± 2^0C).

Enumeration of Bacteria and Fungi Populations

The Total Culturable Heterotrophic Bacteria Counts (THBC) of the POME contaminated soil and amended soil samples were carried out using spread plate method on nutrient agar (NA) (oxoid) (APHA, 1998). Serial ten-

fold dilutions were prepared with normal saline. One gram of soil sample was weighed into test tube containing 9ml normal saline. This was repeated up to 10^{-5}. Aliguots (0.1ml) of 10^{-4} - 10^{-5} dilutions were inoculated onto NA plates in triplicates. The plates were incubated at 37^0C for 24h. The same procedure was used for total fungal (TF) counts, inoculating 1ml of 10^{-4} – 10^{-5} dilutions onto Potato Dextrose Agar (PDA) plates incorporated with lactic acid to inhibit the growth of bacteria. The plates were incubated at 28 ± 2^0C for 3-5 days. Plates were enumerated after incubation periods and expressed as colony forming units per gram (cfu/g).

Isolation and Identification of Bacterial and Fungal Isolates

Culturable bacterial isolates from NA plates were purified by sub-culturing onto NA plates and incubated at 28 ± 2^0C for 24h. Discrete colonies were further sub-cultured onto NA slants in Bijou bottles and incubated at 28 ± 2^0C for 24h. The NA slants were stored in the refrigerator at 40C as pure stock cultures. The pure bacterial isolates were identified based on colonial and cell morphology as well as biochemical characteristics with reference to Bergey and Holt, (1994); Cheesbrough, (2006). Moulds were identified based on macroscopic and microscopic appearances which includes, pigmentation, aerial and substrate hyphae. Isolates were placed on clean and grease free slides with drop of lactphenol and covered with coverslips. The isolates were identified using the scheme of Domsch and Gams (1970) and David *et al.,* (2007).

Determination of Physicochemical Parameters

The physicohemical parameters of the POME contaminated and amended soil samples analysed were pH, Chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total organic content (TOC) respectively. All the parameters were determined using standard laboratory procedures adopted from ASTM (2003) and Stewart *et al.* (1974) the pH was determined using Hach pH Meter (Model ECIO).

RESULTS AND DISCUSSION

The results of total culturable heterotrophic bacterial and total fungal counts of the POME contaminated soil and cow dung are shown in Table 1, while that of the contaminated soil and amended soil during the bioremediation period are shown in Table 2.

Table 1: Total heterotrophic bacterial (THB) and Total Fungal (TF) counts in POME contaminated soil and cow dung samples before bioremediation

Type of Count	PCS	CD
	Values	
THB	$3.0x10^6$	$4.8x10^6$
TF	$2.9x10^4$	$3.6x10^4$

PCS= P.O.M.E Contaminated Soil, CD = Cow Dung, THB=Total Heterotrophic Bacterial count, TF=Total Fungal count

Table 2: Total heterotrophic bacterial (THB) and Total Fungal (TF) counts in POME contaminated soil and contaminated soil amended with cow dung during the bioremediation period

Day	Type of Count	PCS (cfu/g)	PCS + CD (cfu/g)
		Values	
0	THB	$3.0x10^6$	$4.8x10^6$
	TF	$3.2x10^4$	$3.7x10^4$
7	THB	$3.4x10^6$	$5.2x10^6$
	TF	$3.1x10^4$	$4.1x10^4$
14	THB	$3.3x10^6$	$8.3x10^6$
	TF	$3.8x10^4$	$4.8x10^4$

PCS = POME Contaminated Soil, CD=Cow dung, THB=Total Heterotrophic Bacterial count, TF=Total Fungal count.

The physicochemical characteristics of POME contaminated soil before amendment with cow dung and POME contaminated soil amended with cow dung after bioremediation at day 14 is presented in Table 3.

Table 3: Physicochemical characteristics of untreated POME contaminated soil and POME contaminated soil amended with cow dung before and after the bioremediation period.

Parameter	PCS (Before)	PCS+CD (After)
pH	4.16	6.10
COD (mg/kg)	160.10	70.20
BOD (mg/kg)	120.40	90.20
TOC (mg/kg)	140.10	100.10

PCS= Pome Contaminated Soil, PCS+CD=POME contaminated soil amended with Cow dung

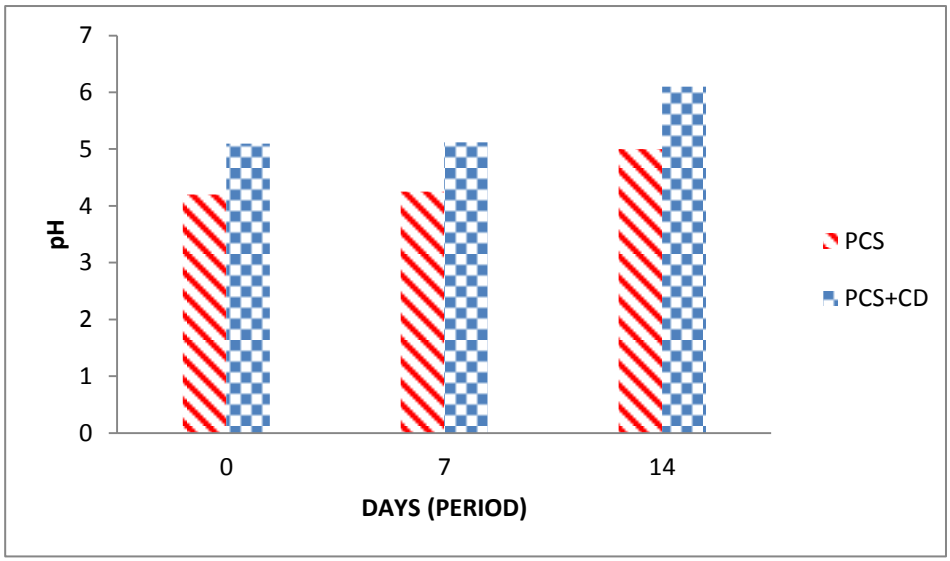

Fig. 1: Changes in pH of POME contaminated soil and POME contaminated soil amended with cow dung during the bioremediation period.

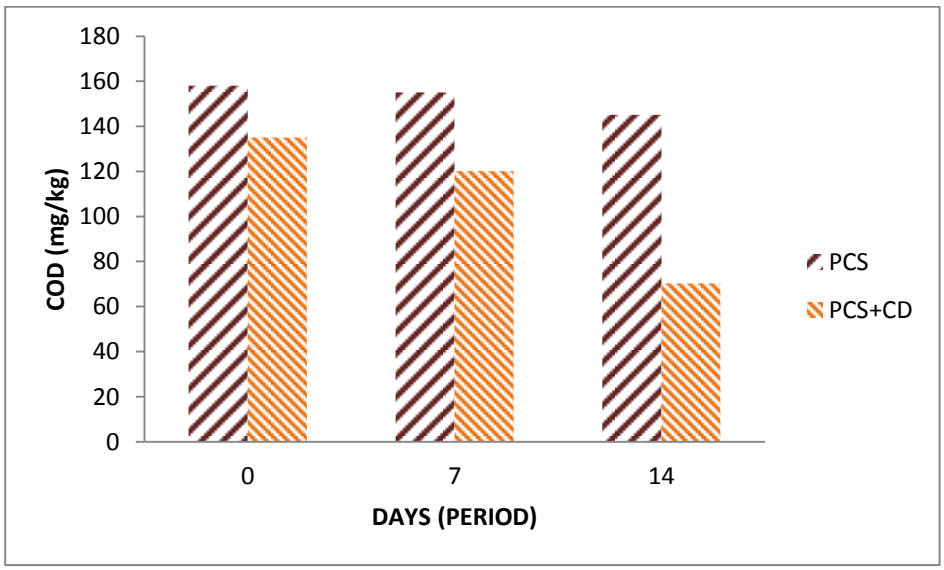

Fig. 2: Changes in COD of POME contaminated soil and POME contaminated soil amended with cow dung during the bioremediation period

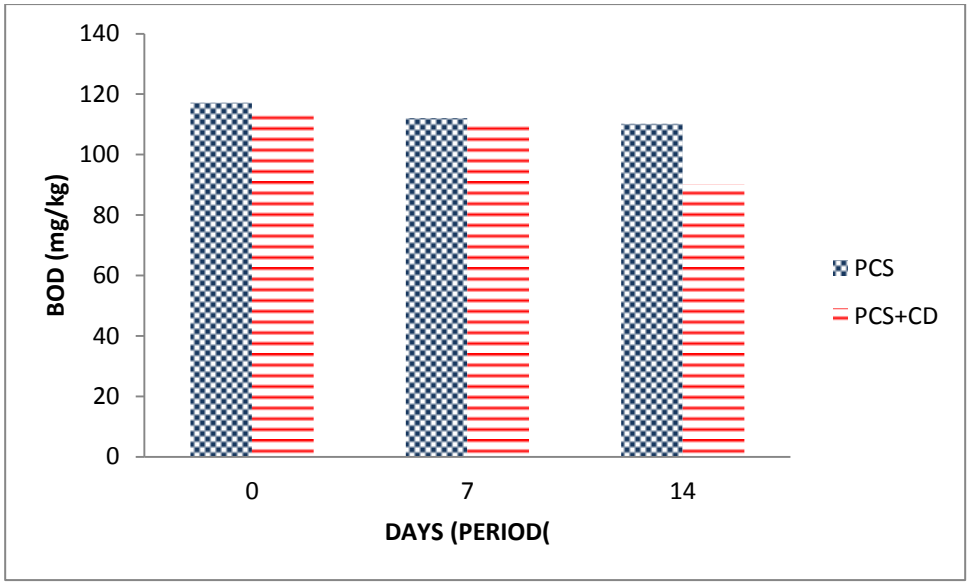

Fig. 3: Changes in BOD of POME Contaminated soil and POME contaminated soil amended with cow dung during the bioremediation period.

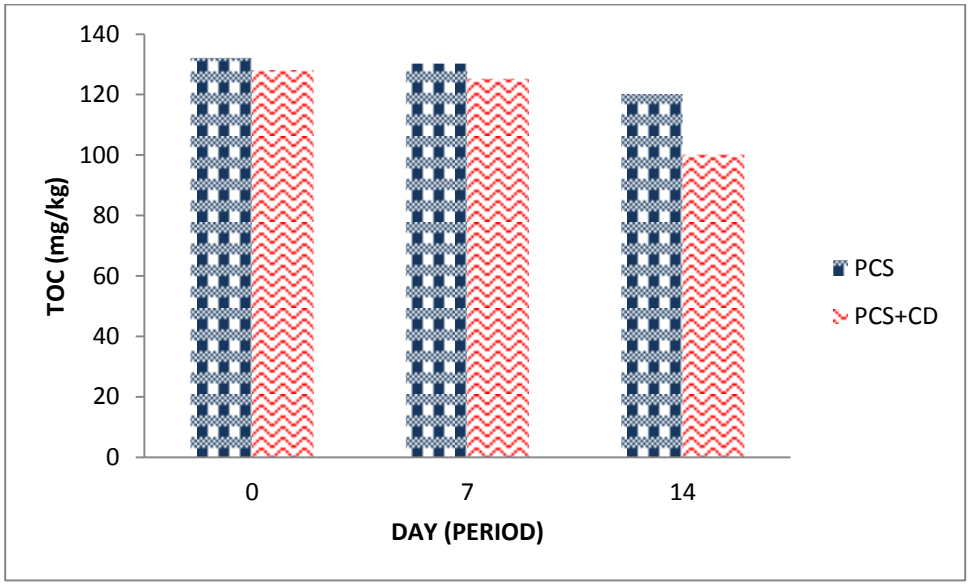

Fig. 4: Changes in TOC in POME contaminated soil and POME contaminated soil amended with cow dung during the bioremediation period.

Changes in physicochemical characteristics of POME contaminated soil and soil amended with cow dung during the bioremediation period are presented in Figs. 1-4. The results showed that there were reductions in COD, BOD and TOC of the POME contaminated soil amended with cow dung while values in pH showed increase from 4.16 to 6.10 indicating reduction in soil acidity Table 3. The high acidic level (pH 4.16) of the POME contaminated soil could be attributed to the acidic nature of POME (Bek-Nielsen *et al.,* 1999). The acidity is as a result of the accumulation of organic acids in the sample due to fermentation process by indigenous microorganisms (Hemming, 1977; Nwaugo *et al.,* 2008; Parveen *et al.,* 2010; Igwe and Onyegbado, 2007; Ibe *et al.,* 2014). The pH value was below the recommended Federal Environmental Protection Agency (FEPA) of Nigeria (1991) effluent limitation guideline of pH 6-9. The values of other physiochemical parameters of the POME

contaminated soil, COD, BOD and TOC showed high values when compared to the POME contaminated soil amended with cow dung during the study period Figs. 1-4. These may be due to the constituents of the POME which include cellulose fruit debris, degradable organic matter and unrecovered palm oil (Ahmed *et al.,* 2003).

The environmental impact of POME on some physiochemical parameters and total aerobic bioload of soil at a dump site in Anyigba Kogi State, Nigeria was investigated by Okwute and Isu (2007). The effect of POME on the integrity of the soil investigated showed significant differences (P≤0.05) and (P≤0.01) in pH water holding capacity, organic carbon, total nitrogen, cation exchange capacity and available phosphorus (Owute and Isu, 2007). From the results of the physicochemical parameters, during the bioremediation period, it showed reductions in COD, BOD and TOC values (Figs. 2-4; Table 3). The reductions in the physicochemical parameters in the amended POME contaminated soil with cow dung was as a result of the high microbial load in the cow dung that enhanced the biodegradation of the organic pollutants in the POME contaminated soil (Owute and Isu, 2007; Owkwute and Ijah, 2014).

The microbial populations of the untreated POME contaminated soil showed low total heterotrophic bacterial (THB) count (3.6 $x10^6$cfu/g) and total fungal (TF) (2.9$x10^4$cfu/g) (Table 1). The microbial populations during the bioremediation period increased from day 0 to day 14. THB (4.8$x10^6$ – 8.3$x10^6$cfu/g) TF count (3.7x 10^4 – 4.8$x10^4$cfu/g) Table 2. The lower bacterial counts recorded in the unamended POME contaminated soil may be attributed to the high acidity and oily content as only microorganism with the competent enzyme systems to proliferate can thrive in it. Fungi tend to thrive well in slightly acidic environments; this could also explain why the fungal and bacterial counts in the unamended POME contaminated soil were nearly the same as bacteria tend not to do well in acidic environments. Benneth and Fasion (1997) attributed the dominance of bacteria degraders to the fact that fungi are more proficient at co-metabolism and bioaccumulation than at using pollutants as sole carbon source, hence the higher THB counts than TF counts throughout the period of bioremediation.

The positive effects of organic nutrient supplement on bioremediation of POME contaminated soil have been reported (Okwute and Ijah, 2014; Bek-Nelson *et al.,* 1999; Obire *et al.,* 2008) these reports agrees with the present study. In the amended soil, with cow dung, the results of THB and TF counts indicated increase from day 0 to day 14 respectively (Table 2).

The results of isolation and identification of bacteria from the amended soil samples to the generic level revealed the following; *Bacillis* species, *Psudomonas* sp., *Micrococcus* sp., *Esherichia coli, Corynebacterium* and Proteus sp, while the fungal genera included *Aspergillus* sp., *Fusarim* sp., *Penicillum* sp., *Geotricum* and *Mucor* sp. The yeast isolated was *Candida* sp.

Similar organisms have been identified in previous studies on bioremediation of POME polluted soil and crude oil polluted soil using microorganism found in organic wastes (Obirre and Akinde, 2008; Obire *et al.,* 2008; Okwute and Ijah, 2014). The present study shows that these isolates (bacteria and fungi) have the degradative ability to degrade the organic pollutants in the POME polluted soil sample.

In conclusion, the findings of this study, suggests that the application of cow dung increased microbial populations in the POME contaminated soil, increased pH values, thereby reducing the acidity and reduction in BOD, COD and TOC of the POME contaminated soil. The bacterial and fungi genera isolated have the potential to degrade the organic pollutants in the POME contaminated soil and can be applied in the ecofriendly technology of clean-up of chemical or hydrocarbon contaminated sites.

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Cite this Article: Wokem, VC; Akpila YI (2021). Bioremediation Of Palm Oil Mill Effluent (POME) Contaminated Soil Using Cow Dung. Greener Journal of Microbiology and Antimicrobials, 6(1): 1-7.