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BIOREMEDIATION OF PALM OIL MILL EFFLUENT (POME) POLLUTED SOIL USING MICROORGANISMS FOUND IN ORGANIC WASTES

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ABSTRACT
The aim of this study was to demonstrate the use of chicken droppings and cow dung in the amendment of soil polluted with palm oil mill effluent (POME) in bioremediation. Soil polluted with 20 % raw (POME) in the laboratory was amended with different concentrations of chicken droppings, cow dung and a combination of the wastes (10 %, 20 % and 30 %). Isolation, characterization and identification of microorganisms were carried out and compared over time with respect to the different concentrations. Gas chromatography mass spectroscopy (GCMS) analysis of extracts of POME polluted and amended soil indicated a reduction in the number of long chain hydrocarbons (C_{13}-C_{44}) in POME polluted soil to C_8-C_{21} in amended soil. This was attributed to the presence of microorganisms of the genera Pseudomonas, Bacillus, Proteus, Micrococcus, Aspergillus, Penicillium, Paecilomyces and Candida in significant numbers throughout the period of analysis. However, a combination of the two organic wastes at 20 % concentration was most effective in this reduction. The implication of these findings is that the bacteria, mould and yeast isolates found in these organic wastes can be useful in rehabilitation of POME polluted soil and possibly other oil polluted sites.

Keywords: Bioremediation, Palm oil mill effluent (POME), Chicken droppings, Cow dung, Organic wastes.

1. INTRODUCTION

Raw palm oil mill effluent (POME) consisting of complex vegetative matter is a 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 (Bek-Nielsen *et al.*, 1999). The raw or partially treated POME usually has an extremely high content of degradable organic matter, which is said to be due in part to the presence of unrecovered palm oil (Ahmad *et al.*, 2003). POME has been reported to alter the physicochemical properties of soil, (Okwute and Isu, 2007), pollution of waterways due to oxygen depletion, (Bek-Nielsen *et al.*, 1999), significantly alter microbial numbers in POME polluted soil (Nwaugo *et al.*, 2008) and reduction in the growth of oil palm seedlings (Nazeeb *et al.*, 1984).

In natural conditions, pollutants are degraded slowly. The implication is that a lot of harm will be done to the ecosystem before such an environment recovers. There is therefore, the need to speed up the rate of recovery of the polluted environment. Bioremediation is any process that uses microorganisms, their enzymes and green plants to return the natural environment altered by contaminants to its original condition (Khan, 2011). Microorganisms alter and break down the oil into other substances such as carbon dioxide, water, and simpler compounds that do not affect the environment. The speed of recovery in a soil environment will greatly depend on the type of contaminants that have been applied to it and for how long. The properties of palm oil mill effluent (POME) which include long chain hydrocarbons in addition to unrecovered oil make it expedient to remedy the polluted soil to hasten the period of recovery of the soil. Bioremediation has been successfully used in the clean-up of petroleum hydrocarbon pollutants (Okoh, 2006), refinery effluents (Ojumu *et al.*, 2005), textile effluents (Bako *et al.*, 2008) and wastewater (Okonko and Shittu, 2007). In an Alaskan oil spill bioremediation study, it was estimated that if an oil spill would normally take five to ten years for natural conditions to return, this could be achieved in as

little as two to five years through the use of bioremediation (Gordon, 1994). Oil palm production in Nigeria has been reported to have risen from 8.2 million tonnes in 1990 to 9 million metric tonnes in 2001 (Food and Agricultural Organization (FAO), 2002).

About 43-45 % of this is always a mill residue in the form of Empty Fruit Bunches (EFB), Shell, Fibre and Palm Oil Mill Effluent (POME) (Nwoko and Ogunyemi, 2010). These residues are expected to continue to accumulate with increasing production.

Chicken droppings and cow dung are readily available in the environment as wastes and they are cheap to obtain. The presence of a variety of microorganisms in chicken droppings and cow dung with the ability of breaking down oil helps to speed up the process of bioremediation.

Besides, these wastes have been found to be rich in nitrogen and phosphorus (Ijah and Antai, 2003), which are crucial in the biodegradation of organic pollutants. The aim of this study is to demonstrate the ability of microorganisms found in organic wastes to significantly reduce organic compounds in palm oil mill polluted soil sites thereby bringing about bioremediation.

2. MATERIALS AND METHODS

2.1. Collection of Samples

2.1.1. Soil Samples

The soil samples used for bioremediation studies were collected from a demarcated area within the Kogi State University, Faculty of Agriculture farmland at Anyigba, bulked into a composite sample, poured into properly labeled clean polythene bags and transported from the site to the laboratory. The samples were air-dried, stones and unwanted materials were removed and the residue crushed to finer particles to ensure passage through a 2 mm mesh before use.

2.1.2. Palm Oil Mill Effluent (POME)

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 or barrel, was mixed thoroughly before being fetched 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^{0}C.

2.1.3. Organic Wastes

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-Abuja 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. Experimental Design and Treatment

The pollution of soil with POME was done using a simple randomized block design with three replicates. Each treatment represented a block with three plastic buckets for the three replicates. 33 buckets were filled with soil from the 0-30 cm of top soil weighing 6 kg per bucket from the study area. 30 buckets were polluted with POME at a moderate pollution level of 20 %. (This translated to 1200 ml of POME being applied to 6 kg of soil). Bioremediation was done with chicken droppings, cow dung and a combination of the two at three different concentrations (10 %, 20 % and 30 %) in triplicates. Three buckets containing POME and soil and three buckets containing soil alone had no organic waste applied to them, and served as control. Sampling was done immediately after pollution and subsequently, after one month and two months.

2.3. Microbiological Analyses

Microorganisms in the soil samples were enumerated by spread inoculating 0.1ml ten-fold serially diluted samples onto nutrient agar (NA), Sabouraud Dextrose agar (SDA) and Palm oil agar (POA) for the enumeration of aerobic heterotrophic bacteria, fungi and palm oil utilizing bacteria respectively. The POA contained 10 ml of palm oil in 990 ml of mineral salts medium (Zajic and Suplisson, 1972) containing K_2HPO_4, 1.8g/l, KH_2PO_4, 1.2g/l, NH_4Cl, 4.0g/l, Mg $SO_4.7H_20$, 0.2g/l, $FeSO_4.7H_2O$, 0.01g/l, NaCl, 0.1g/l, agar-agar, 20g/l and 1 % palm oil. The

inoculated NA plates were incubated at 30 ^{0}C for 48 h while the SDA plates were incubated at 25 ^{0}C for 3-5 days. Observed colonies were counted and expressed as colony forming units per gram (cfu g^{-1}) of soil.

2.3.1. Characterization and Identification of Microbial Isolates

2.3.1.1. Bacterial Isolates

The bacterial isolates were characterized based on their cultural and biochemical properties which included production of coagulase, catalase, indole, urease, motility test, citrate utilization test, starch hydrolysis, Methyl Red-Voges Proskaeur (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 by Buchanan and Gibbons (1974) in Bergey's Manual of Determinative Bacteriology.

2.3.1.2. 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 Domsch and Gams (1970).

2.3.1.3. Yeast Isolates

Yeast isolates were Gram stained and characterized based on colonial morphology, cell micromorphology, germ tube and blastosphore formation, gelatin liquefaction, starch hydrolysis, growth at 37 ^{0}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 Barnett and Pankhurst (1974).

2.4. Gas Chromatography-Mass Spectroscopy (GC-MS) of Palm Oil Mill Effluent and Soil Extracts

To obtain the POME extract, 1 litre of the POME was extracted with one litre of petroleum ether (40-60 ^{0}C) two consecutive times and evaporated to dryness. For the soil samples, 100 g of each was extracted using a Soxhlet extractor (Electrothermal) with 500 cm^3 of petroleum ether (40-60 ^{0}C) and evaporated to dryness. Gas chromatography (GC) analysis of the extracts was carried out using GC-MS-QP2010 PLUS (Shimadzu, Japan) which was equipped with a capillary inlet and mass selective detector set to scan from m z^{-1} 45 to m z^{-1} 800 at a scan rate of 1.2 scans second^{-1}. The injection temperature was programmed from 80 ^{0}C to 250 ^{0}C at a total flow rate of 6.2 mL min^{-1} using helium as the carrier gas.

3. STATISTICAL ANALYSIS

Data generated from the study were analyzed using the computer package SPSS (Version 19.0) (Statistical Package for Social Sciences (SPSS), 2010) which employed the use of univariate analysis of variance (ANOVA) at the $P \leq 0.05$ confidence limit to analyse the variance in the values obtained from all laboratory analyses.

4. RESULTS

4.1. Physicochemical Properties

The physicochemical properties of unpolluted soil, cow dung, chicken droppings and palm oil mill effluent (POME) are presented in Table 1. Cow dung had the highest pH (8.64) while the unpolluted soil had the highest percentage moisture (7.03 %). Chicken droppings had the highest value for nitrogen (0.31 %) while POME had the lowest (0.03 %). The unpolluted soil had the highest available phosphorus (21.40 mg kg^{-1}) while chicken droppings had the highest mineral assay values with calcium being the highest with 57.2 mg l^{-1}.

4.2. Microbial Counts

In Table 2 which presents the microbial counts in unpolluted soil, chicken droppings, cow dung and POME, chicken droppings had the highest counts in bacteria ($5.6 \times 10^9 \pm 0.09$ cfu g^{-1}), moulds/yeasts ($3.8 \times 10^5 \pm 0.12$ cfu g^{-1}) and palm oil utilizing microorganisms ($4.4 \times 10^5 \pm 0.1$ cfu g^{-1}) while POME had the lowest values ($4.0 \times 10^9 \pm 0.12$ cfu ml^{-1}) for bacteria, ($2.6 \times 10^3 \pm 0.06$ cfu ml^{-1}) for moulds/yeasts and ($2.6 \times 10^3 \pm 0.12$ cfu ml^{-1}) for palm oil mill utilizing microorganisms.

In Table 3, the bacterial counts in the different soil samples were significant at $P \leq 0.05$ confidence limit for chicken droppings 30 % with a value of $8.5 \times 10^7 \pm 0.12$ cfu g^{-1} and cow dung + chicken droppings 20 % with a value of $8.7 \times 10^7 \pm 0.02$ cfu g^{-1}. Table 4 shows the counts for moulds being significant in cow dung + chicken droppings 20 % with a value of $8.9 \times 10^3 \pm 0.004$ cfu g^{-1} and cow dung + chicken droppings 30 % with a value of $9.0 \times 10^3 \pm 0.007$ cfu g^{-1}. In Table 5, yeast counts were significant ($P \leq 0.05$) for chicken droppings 30 % with a value of $7.0 \times 10^3 \pm 0.03$ cfu g^{-1} and cow dung + chicken droppings 20 % with a value of $7.3 \times 10^3 \pm 0.01$ cfu g^{-1}.

4.3. Occurrence of Microorganisms

In Table 6, *Pseudomonas aeruginosa*, *Bacillus* spp. and *Micrococcus roseus* occurred most frequently while *Staphylococcus aureus* was absent for most part of the observations. However, all the bacteria were present in chicken droppings (30 %) and chicken droppings + cow dung (30 %) throughout the period of bioremediation. In Table 7, *Penicillium verrucossum* was present in all treatments while *Fusarium sp.* occurred least frequently. Table 8 shows *Candida albicans* occurring most frequently and *Saccharomyces cerevisiae* occurring least frequently.

4.4. GC-MS Chromatograms

Table 9 shows a summary of the chromatograms of the unpolluted and amended soil samples and the depletion in organic compounds over the period of bioremediation. Cow dung + Chicken droppings (20 %) had the lowest number of organic compounds (6 peaks) when compared to that of the POME polluted unamended soil which had 17 peaks. This translated to a 33.3% reduction in number of organic components when compared to POME polluted soil.

5. DISCUSSION

The pH of palm oil mill effluent (POME) used was 4.16 (Table 1). This attributed to the acidic nature of raw POME (Bek-Nielsen *et al.*, 1999). The value was below the Federal Environmental Protection Agency, Nigeria Federal Environmental Protection Agency (FEPA) (1991) effluent limitation guideline of 6-9. Other physicochemical properties such as moisture, organic matter/carbon, nitrogen, available phosphorus and mineral assay were all low when compared to that of soil, chicken droppings and cow dung. This may be due to the constituents of the POME which include cellulose fruit debris, degradable organic matter and unrecovered palm oil. However, the chicken droppings had higher organic matter/carbon, available phosphorus and nitrogen when compared to cow dung though the pH of cow dung was higher (8.64). These properties of the organic wastes made them good agents for bioremediation.

The chicken droppings used had high counts (5.6×10^9cfu g^{-1}) of bacteria, fungi (3.8×10^3cfu g^{-1}) and oil utilizing bacteria (4.4×10^5cfu g^{-1}) (Table 2). These counts were higher than those reported by Obire and Akinde (2008) and Obire *et al.* (2008). The difference in counts could be due to higher pH and organic matter content which could aid the proliferation of microorganisms. The POME had counts of 4.0×10^9cfu ml^{-1}, 2.6×10^3cfu ml^{-1} and 2.6×10^3cfu ml^{-1} for bacteria, fungi and oil utilizing bacteria respectively. The microbial counts for soil were 4.2×10^9cfu g^{-1} for bacteria, 3.0×10^3cfu g^{-1} for fungi and 5.4×10^3cfug^{-1} for oil utilizing bacteria. These counts were similar to those reported by Ijah *et al.* (2008). The lower counts recorded in the POME may be attributed to its acidic and oily content as only microorganisms with the competent enzyme systems to proliferate can be found in it.

The bacteria, mould and yeast counts were most significant ($P \leq 0.05$) at chicken droppings (30 %) and cow dung + chicken droppings (20 %), cow dung + chicken droppings (20 and 30 %) and chicken droppings (30 %) and cow dung + chicken droppings (20 %) respectively (Tables 3, 4 and 5). This observation is corroborated by the chromatograms which show a reduction from 17 peaks in POME polluted soil to 6 peaks in soil amended with cow dung + chicken droppings (20

%). *Pseudomonas aeruginosa* and *Bacillus* species (*B. licheniformis and B. subtilis*) (Table 6) were consistently isolated in the polluted and amended soils. The isolation of species of *Pseudomonas*, *Bacillus* and *Proteus* in amended soil had previously been reported (Olajide and Ogbeifun, 2010).

The occurrence of *Pseudomonas aeruginosa* in POME polluted soil may be due to their ability to utilize oil as their carbon source (Sira *et al*., 2010). Pseudomonads are the best known bacteria capable of utilizing hydrocarbons as carbon and energy sources and producing biosurfactants when grown on carbon sources (Cameotra and Singh, 2008). This may have also been the reason for their presence in the soil even after 2 months of bioremediation of POME polluted soil.

The moulds most frequently isolated from the amended polluted soil in the laboratory were genera of *Aspergillus*, *Trichophyton*, *Paecilomyces*, and *Penicillium* (Table 7). The breakdown of petroleum hydrocarbons by moulds particularly of the genera *Aspergillus*, *Trichoderma*, *Penicillium*, *Mucor* and *Fusarium* has been reported by several authors (Obire *et al*., 2008; Ibiene *et al*., 2011). *Aspergillus* sp. in particular are reported to be good producers of cellulases; the enzymes responsible for the breakdown of cellulose in POME (Wong *et al*., 2008). The yeasts that occurred most frequently were *Candida albicans*, and *Rhodotorula rubra* (Table 8). The utilization of hydrocarbons by yeasts as carbon source particularly *Candida* sp., *Saccharomyces* sp., *Torulopsis candida* and *Rhodotorula* sp. was reported by Obire *et al*. (2008) and Omotayo *et al*. (2011). Fungi are notably aerobic and can also grow under environmentally stressed conditions such as low pH and poor nutrient status (Davis and Westlake, 1979). These are conditions which were brought about in the POME polluted soil by the properties of POME.

An analysis of the summary table of the chromatograms of POME extracts (Table 9) from the polluted and the amended soils showed that the soil treatment whose organic compounds had been most depleted in the laboratory was the combination of chicken droppings and cow dung (20 %) which had the most reduced number of hydrocarbons after 2 months bioremediation. This combination also had the most significant ($P \leq 0.05$) microbial counts. In addition, the (GC) analyses of the soil extracts indicated a reduction in the number of long chain hydrocarbons from 17 peaks (C_{13}-C_{44}) in POME polluted soil to 6 peaks (C_8-C_{21}). There was also a general reduction in the intensity of the peaks. This shows that most of the compounds responsible for the pollution of the soil by the POME had been used by the microorganisms resident in the soil and in the organic wastes. This indicates that this organic waste combination is very useful as a bioremediation agent as the organic component peaks had decreased in number and intensity.

6. CONCLUSION

From the results of this study, the chicken droppings and cow dung and the microorganisms resident in them were effective agents in the reduction of levels of POME in soil. In addition, a combination of the two organic wastes at 20 % concentration was most effective in the reduction in the number and intensity of long chain hydrocarbons present in POME polluted soil samples. The implication of these findings is that the bacteria, mould and yeast isolates can be useful in an environmentally friendly way of rehabilitating POME polluted soil and possibly other oil polluted sites. The choice of cow dung and chicken droppings is because of their abundant availability in Nigeria thus making them cheaper to use than physical and chemical methods available.

Table-1. Physicochemical properties of unpolluted Soil, Organic wastes (chicken droppings and cow dung) and POME

Parameters	Soil	Cow dung	Chicken droppings	POME	FEPA effluent limitation Guideline (1991) Mg l^{-1}
pH	6.93±0.1	8.64±0.2	6.89±0.1	4.16±0.1	6-9
Moisture (%)	7.03±0.2	5.30±0.01	1.32±0.01	NA	NA
Org. Matter (%)	0.98±0.02	33.72±0.1	76.22±0.3	1.19±0.01	NA
Org. Carbon (%)	0.57±0.02	19.50±0.1	44.08±0.05	0.69±0.01	NA
Nitrogen (%)	0.08± 0.01	0.15± 0.01	0.31± 0.01	0.03± 0.01	10
Available P	21.4± 0.1	0.61± 0.02	1.02± 0.01	0.02±0.001	NA
Na^+	0.26± 0.01	4.0± 0.2	8.35± 0.1	1.65± 0.01	NA
K^+	3.5± 0.1	0.36± 0.01	30.77± 0.5	1.64± 0.01	NA
Ca^{2+}	9.7± 0.1	29.60± 0.3	57.20± 0.5	8.16±0.02	75
Mg^{2+}	3.8±0.04	14.80±0.3	45.60± 0.5	2.45±0.01	50
ECEC	17.26±0.3	NA	NA	NA	NA
TDS (mg l^{-1})	NA	NA	NA	1549.0±1	2000
BOD (mg l^{-1})	NA	NA	NA	15.80±0.33	500
COD (mg l^{-1})	NA	NA	NA	24.80±0.02	40
Oil & grease(mg l^{-1})	NA	NA	NA	10.0±0.01	30
Heavy metals (ppm)					
Copper	0.20±0.01	NA	NA	0.49±0.01	1.0
Lead	0.43±0.02	NA	NA	1.70±0.01	0.05
Iron	22.03±0.2	NA	NA	33.40±0.4	20
Zinc	0.05	NA	NA	0.18	1.00

ECEC=Effective Cation Exchange Capacity, POME—Palm Oil Mill Effluent, NA------Not Applicable or none set, Federal Environmental Protection Agency (FEPA) (1991).

Table-2. Microbial Counts in Soil, Palm Oil Mill Effluent and Organic Wastes Used

Sample	Bacteria	Moulds/Yeasts	POU m/orgs
Soil (cfu g^{-1})	$4.2 \times 10^9 \pm 0.15$*	$3.0 \times 10^3 \pm 0.15$	$5.4 \times 10^3 \pm 0.2$
POME (cfu ml^{-1})	$4.0 \times 10^9 \pm 0.12$	$2.6 \times 10^3 \pm 0.06$	$2.6 \times 10^3 \pm 0.12$
Chicken droppings (cfu g^{-1})	$5.6 \times 10^9 \pm 0.09$	$3.8 \times 10^5 \pm 0.12$	$4.4 \times 10^5 \pm 0.1$
Cow dung (cfu g^{-1})	$5.1 \times 10^9 \pm 0.14$	$2.8 \times 10^3 \pm 0.12$	$3.5 \times 10^4 \pm 0.09$

*Values are means of three replicates + SEM
POME= Palm oil mill effluent, POU m/orgs= Palm oil utilizing microorganisms,

Table-3. Total Bacterial Counts in palm oil mill effluent (POME) and amended Soil Samples after Two Months of Bioremediation

Treatment	Bacterial counts (cfu g^{-1}) Time (Months) 0	1	2
A	$1.3 \times 10^4 \pm 0.04$	$4.0 \times 10^5 \pm 0.17$	$5.2 \times 10^6 \pm 0.01$
B	$2.5 \times 10^4 \pm 0.06$	$5.1 \times 10^5 \pm 0.02$	$6.8 \times 10^6 \pm 0.04$
C	$3.4 \times 10^4 \pm 0.71$	$5.8 \times 10^5 \pm 0.15$	$7.5 \times 10^6 \pm 0.02$
D	$1.8 \times 10^4 \pm 0.56$	$4.5 \times 10^5 \pm 0.11$	$6.7 \times 10^6 \pm 0.11$
E	$2.7 \times 10^5 \pm 0.66$	$5.5 \times 10^5 \pm 0.20$	$7.0 \times 10^6 \pm 0.03$
F	$3.8 \times 10^6 \pm 0.23$	$7.5 \times 10^6 \pm 0.01$	$8.5 \times 10^7 \pm 0.12$*
G	$2.3 \times 10^4 \pm 0.01$	$4.7 \times 10^5 \pm 0.09$	$6.4 \times 10^6 \pm 0.01$
H	$3.1 \times 10^4 \pm 0.03$	$4.0 \times 10^5 \pm 0.34$	$8.7 \times 10^7 \pm 0.02$*
I	$4.4 \times 10^4 \pm 0.13$	$6.3 \times 10^5 \pm 0.05$	$7.5 \times 10^6 \pm 0.01$
J	$2.8 \times 10^4 \pm 0.21$	$3.0 \times 10^5 \pm 0.12$	$3.5 \times 10^5 \pm 0.15$
K	$4.2 \times 10^6 \pm 0.15$	$4.8 \times 10^6 \pm 0.01$	$4.5 \times 10^6 \pm 0.01$

Values are means of three replicates + SEM
*----Significant at $P < 0.05$ confidence limit
A=Cow dung 10 %, B=Cow dung 20 %, C=Cow dung 30 %, D=Chicken droppings 10 %, E=Chicken droppings 20 %, F=Chicken droppings 30 %, G=Cow dung +Chicken droppings 10 %, H=Cow dung + Chicken droppings 20 %, I=Cow dung + Chicken droppings 30 %, J= Polluted unamended soil, K= Unpolluted soil.

Table-4. Total Mould Counts in palm oil mill effluent (POME) Soil Samples after Two Months of Bioremediation

Treatment	Mould counts (cfu g^{-1})		
	Time (Months)		
	0	1	2
A	$1.7 \times 10^3 \pm 0.01$	$2.1 \times 10^3 \pm 0.005$	$3.2 \times 10^3 \pm 0.002$
B	$1.6 \times 10^3 \pm 0.02$	$3.3 \times 10^3 \pm 0.02$	$4.3 \times 10^3 \pm 0.015$
C	$1.9 \times 10^3 \pm 0.01$	$2.5 \times 10^3 \pm 0.001$	$4.7 \times 10^3 \pm 0.008$
D	$1.0 \times 10^3 \pm 0.002$	$3.0 \times 10^3 \pm 0.02$	$5.4 \times 10^3 \pm 0.005$
E	$1.3 \times 10^3 \pm 0.001$	$3.4 \times 10^3 \pm 0.001$	$5.3 \times 10^3 \pm 0.001$
F	$2.3 \times 10^3 \pm 0.04$	$6.0 \times 10^3 \pm 0.02$	$7.5 \times 10^3 \pm 0.001$
G	$3.1 \times 10^3 \pm 0.02$	$5.6 \times 10^3 \pm 0.01$	$7.0 \times 10^3 \pm 0.006$
H	$4.3 \times 10^3 \pm 0.01$	$6.0 \times 10^3 \pm 0.003$	$8.9 \times 10^3 \pm 0.004$*
I	$5.5 \times 10^3 \pm 0.03$	$7.0 \times 10^3 \pm 0.01$	$9.0 \times 10^3 \pm 0.007$*
J	$3.0 \times 10^2 \pm 0.03$	$5.0 \times 10^2 \pm 0.001$	$5.4 \times 10^2 \pm 0.001$
K	$2.0 \times 10^3 \pm 0.01$	$7.0 \times 10^3 \pm 0.001$	$6.0 \times 10^3 \pm 0.001$

Values are means of three replicates ± SEM
*----Significant at P ≤ 0.05 confidence limit
A=Cow dung 10 %, B=Cow dung 20 %, C=Cow dung 30 %, D=Chicken droppings 10 %, E=Chicken droppings 20 %, F=Chicken droppings 30 %, G=Cow dung + Chicken droppings 10 %, H=Cow dung + Chicken droppings 20 %, I=Cow dung + Chicken droppings 30 %, J= Polluted unamended soil, K= Unpolluted soil.

Table-5. Total Yeast Counts in palm oil mill effluent (POME) Soil Samples after Two Months of Bioremediation

Treatment	Yeast counts (cfu g^{-1})		
	Time (Months)		
	0	1	2
A	No detectable growth	$1.8 \times 10^2 \pm 0.03$	$2.0 \times 10^2 \pm 0.003$
B	No detectable growth	$1.6 \times 10^2 \pm 0.01$	$2.2 \times 10^2 \pm 0.02$
C	$1.3 \times 10^3 \pm 0.01$	$2.4 \times 10^3 \pm 0.001$	$3.5 \times 10^3 \pm 0.001$
D	$1.8 \times 10^3 \pm 0.03$	$3.2 \times 10^3 \pm 0.01$	$4.7 \times 10^3 \pm 0.012$
E	$2.6 \times 10^3 \pm 0.002$	$6.5 \times 10^3 \pm 0.04$	$1.0 \times 10^3 \pm 0.02$
F	$3.7 \times 10^3 \pm 0.001$	$5.8 \times 10^3 \pm 0.01$	$7.0 \times 10^3 \pm 0.03$*
G	$1.5 \times 10^3 \pm 0.02$	$3.9 \times 10^3 \pm 0.01$	$6.0 \times 10^3 \pm 0.03$
H	$2.9 \times 10^3 \pm 0.01$	$4.1 \times 10^3 \pm 0.02$	$7.3 \times 10^3 \pm 0.01$*
I	$3.5 \times 10^3 \pm 0.02$	$5.2 \times 10^3 \pm 0.01$	$6.5 \times 10^3 \pm 0.02$
J	No detectable growth	$1.3 \times 10^2 \pm 0.002$	$1.5 \times 10^2 \pm 0.01$
K	$1.0 \times 10^3 \pm 0.02$	$1.4 \times 10^3 \pm 0.01$	$1.2 \times 10^3 \pm 0.02$

Values are means of three replicates ± SEM
*--- Significant at P ≤ 0.05 confidence limit
A=Cow dung 10 %, B=Cow dung 20 %, C=Cow dung 30 %, D=Chicken droppings 10 %, E=Chicken droppings 20 %, F=Chicken droppings 30 %, G=Cow dung + Chicken droppings 10 %, H=Cow dung + Chicken droppings 20 %, I=Cow dung + Chicken droppings 30 %, J= Polluted unamended soil, K= Unpolluted soil.

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

		Bacterial Isolates																	
Treatment	Time	*Pseudomonas aeruginosa*			*Bacillus* sp.			*Staphylococuus aureus*			*Escherichia coli*			*Proteus vulgaris*			*Micrococcus roseus*		
(months)		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 10 %, B=Cow dung 20 %, C=Cow dung 30 %, D=Chicken droppings 10 %, E=Chicken droppings 20 %, F=Chicken droppings 30 %, G=Cow dung + Chicken droppings 10 %, H=Cow dung + Chicken droppings 20 %, I=Cow dung + Chicken droppings 30 %, J=Polluted unamended soil, K=Unpolluted soil, + = Presence of bacteria, - = Absence of bacteria.

Table-7. Occurrence of Moulds in Amended Palm Oil Mill Effluent Polluted Soil

		Mould Isolates																				
Treatment	Time	*Aspergillus niger variotii*			*Mucor mucedo*			*Penicillium verrucossum*			*Fusarium* sp.			*Trichophyton* sp.			*Rhizopus oryzae*			*Paecilomyces*		
	(months)	0	1	2	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 10 %, B=Cow dung 20 %, C=Cow dung 30 %, D=Chicken droppings 10 %, E=Chicken droppings 20 %, F=Chicken droppings 30 %, G=Cow dung + Chicken droppings 10 %, H=Cow dung + Chicken droppings 20 %, I=Cow dung + Chicken droppings 30 %, J=Polluted unamended soil, K=Unpolluted soil, + = Presence of moulds, - = Absence of moulds.

Table-8. Occurrence of Yeasts in Amended Palm Oil Mill Effluent Polluted Soil

	Yeast Isolates											
	Candida albicans			*Saccharomyces cerevisiae*			*Torulopsis candida*			*Rhodotorula rubra*		
Treatment	**Time (months)**	**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 10 %, B=Cow dung 20 %, C=Cow dung 30 %, D=Chicken droppings 10 %, E=Chicken droppings 20 %, F=Chicken droppings 30 %, G=Cow dung + Chicken droppings 10 %, H=Cow dung + Chicken droppings 20 %, I=Cow dung + Chicken droppings 30 %, J=Polluted unamended soil, K=Unpolluted soil, + = Presence of yeasts, - = Absence of yeasts.

Table-9. Compounds detected in POME extracted from amended polluted soil after 2 months

Soil samples	Number of Peaks and Retention time (mins) /Number of C atoms																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
POME	6.065 (C_9)	7.742 (C_6)	8.342 (C_8)	16.108 (C_7)	25.133 (C_{15})	27.967 (C_{18})	28.375 (C_{18})	29.167 (C_{19})	29.525 (C_{16})	29.775 (C_{16})	30.850 (C_{18})	34.500 (C_{30})	22.275 (C_{14})							
UPS	21.908 (C_{13})	23.773 (C_{14})	24.914 (C_{17})	26.806 (C_{19})	29.563 (C_{19})	30.167 (C_{22})	30.408 (C_{21})	30.717 (C_{19})	31.497 (C_{24})	32.008 (C_{20})	32.817 (C_{44})	33.700 (C_{20})								
PS0L	26.574 (C_{16})	27.844 (C_{44})	28.181 (C_{16})	28.941 (C_{17})	29.265 (C_{20})	29.509 (C_{24})	30.140 (C_{16})	30.516 (C_{28})	30.708 (C_{21})	31.313 (C_{20})	31.805 (C_{44})	31.909 (C_{21})	32.084 (C_{29})	32.763 (C_{13})	33.639 (C_{24})	34.029 (C_{29})	34.764 (C_{34})			
A	3.063 (C_{10})	3.464 (C_8)	27.670 (C_{17})	27.948 (C_{16})	28.802 (C_{19})	29.118 (C_{18})	29.264 (C_{39})	29.904 (C_{15})	31.025 (C_{16})	34.075 (C_{15})										
B	25.237 (C_{17})	25.562 (C_{17})	26.156 (C_{18})	26.503 (C_{18})	26.986 (C_{19})	27.664 (C_{11})	27.933 (C_{16})	28.846 (C_{20})	29.015 (C_{19})	29.096 (C_{16})	29.257 (C_{21})	29.505 (C_{17})	30.133 (C_{23})	30.700 (C_{24})	31.307 (C_{18})	31.982 (C_{20})	32.758 (C_{27})	33.668 (C_{21})	34.758 (C_{34})	
C	3.151 (C_{10})	3.462 (C_8)	3.660 (C_8)	27.666 (C_{17})	28.847 (C_{19})	29.101 (C_{16})	31.447 (C_{24})													
D	3.460 (C_9)	27.666 (C_{17})	27.944 (C_{16})	28.144 (C_{18})	28.848 (C_{19})	29.018 (C_{19})	29.263 (C_{14})	29.433 (C_{20})	30.137 (C_{21})	30.704 (C_{21})	31.319 (C_{21})	31.473 (C_{24})	31.933 (C_{21})							
E	3.156 (C_8)	3.467 (C_8)	3.666 (C_8)	27.959 (C_{16})	29.132 (C_{16})	29.268 (C_{37})	31.183 (C_{19})	32.233 (C_{15})	32.919 (C_{34})											
F	3.454 (C_8)	26.500 (C_{14})	26.825 (C_{10})	28.129 (C_{16})	29.263 (C_{14})	29.508 (C_{20})	30.136 (C_{17})	30.702 (C_{28})	31.449 (C_{24})	32.146 (C_{29})	32.765 (C_{27})	34.033 (C_{44})	34.771 (C_{34})							
G	23.849 (C_{10})	25.240 (C_{17})	25.566 (C_{17})	25.919 (C_{17})	26.201 (C_{18})	26.507 (C_{18})	26.824 (C_{18})	27.667 (C_{17})	28.849 (C_{19})	29.016 (C_{19})	30.135 (C_{24})	30.700 (C_{21})	31.445 (C_{24})	34.761 (C_{21})						
H	3.464 (C_8)	3.663 (C_8)	4.030 (C_8)	29.263 (C_{14})	30.516 (C_{16})	34.767 (C_{21})														
I	3.457 (C_8)	3.655 (C_8)	27.940 (C_{16})	29.260 (C_{14})	29.506 (C_{20})	30.515 (C_{28})	30.702 (C_{24})	31.309 (C_{28})	31.908 (C_{54})	32.762 (C_{21})										

POME=Palm oil mill effluent, UPS= Unpolluted soil, PS0L= Polluted unamended soil (time zero), A=Cow dung 10 %, B=Cow dung 20 %, C=Cow dung 30 %, D=Chicken droppings 10 %, E=Chicken droppings 20 %, F=Chicken droppings 30 %, G=Cow dung + Chicken droppings 10 %, H=Cow dung + Chicken droppings 20 %, I=Cow dung + Chicken droppings 30 %.

Figure-1. GC-MS of Palm Oil Mill Effluent Extract

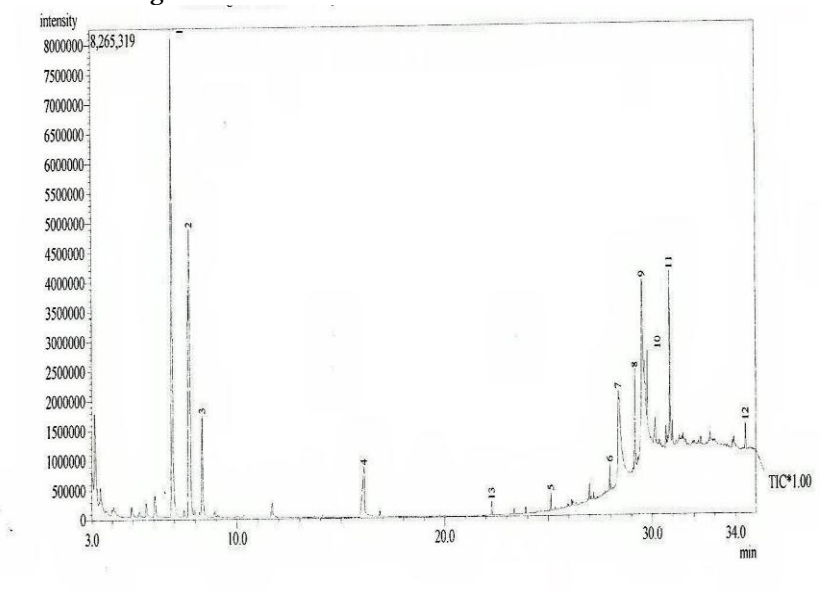


Figure-2. GC-MS of Unpolluted Soil Extract

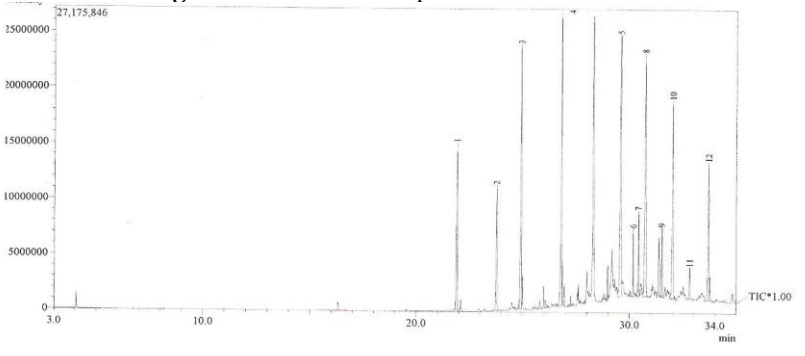


Figure-3. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract at Time Zero of Pollution

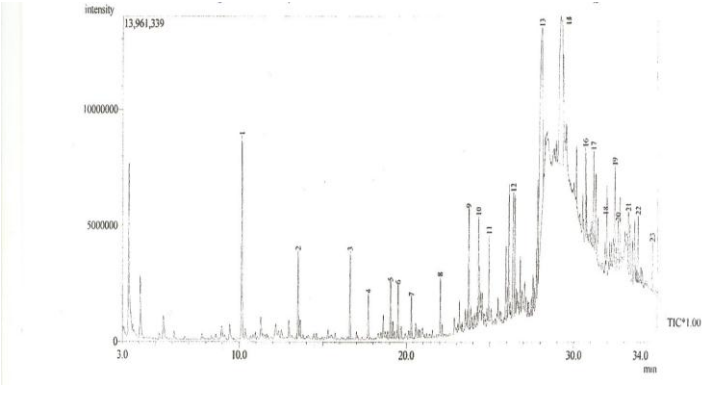


Figure-4. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with Cow Dung (10) Concentration for Two Months

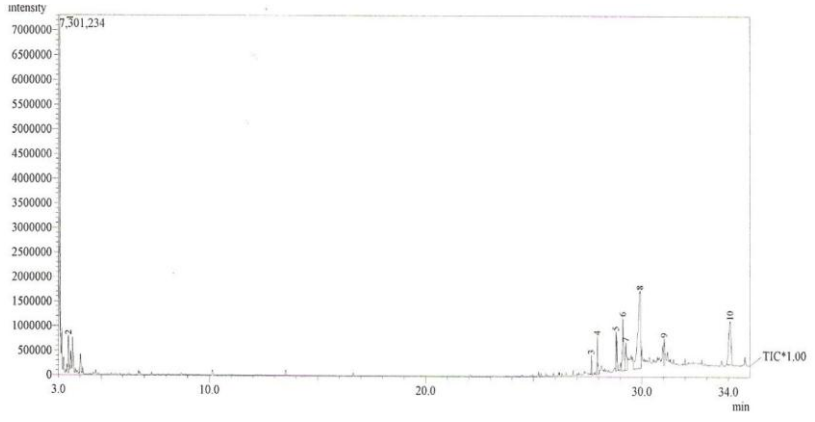


Figure-5. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with Cow Dung (20%) Concentration for Two Months

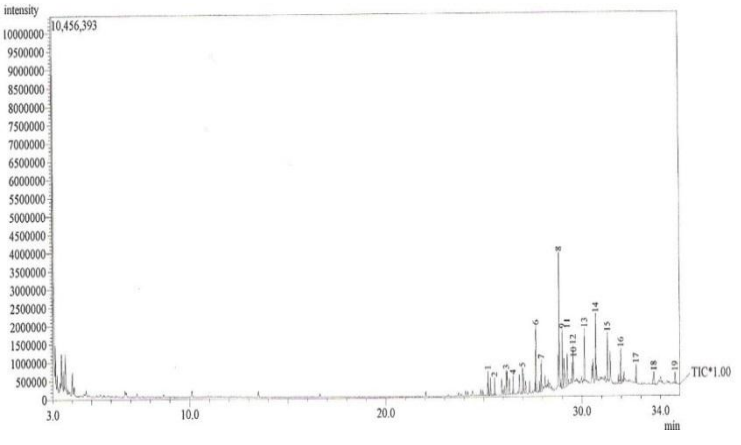


Figure-6. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with Cow Dung (30 %) Concentration for Two Months

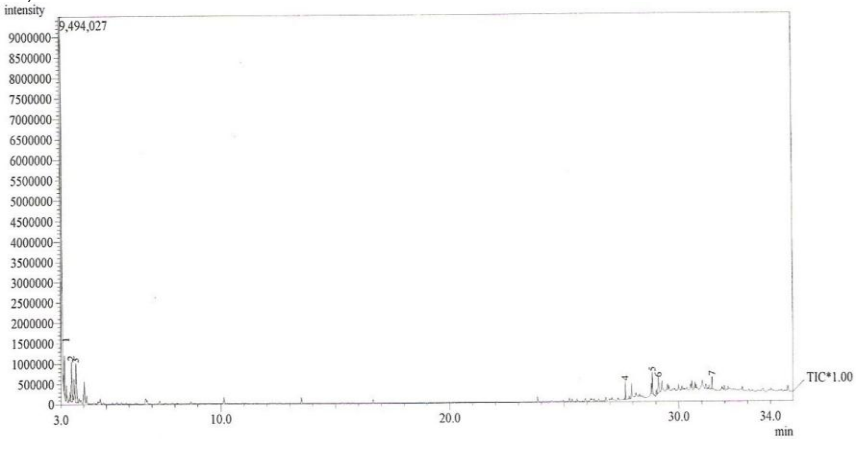


Figure-7. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with Chicken Droppings (10 %) Concentration for Two Months

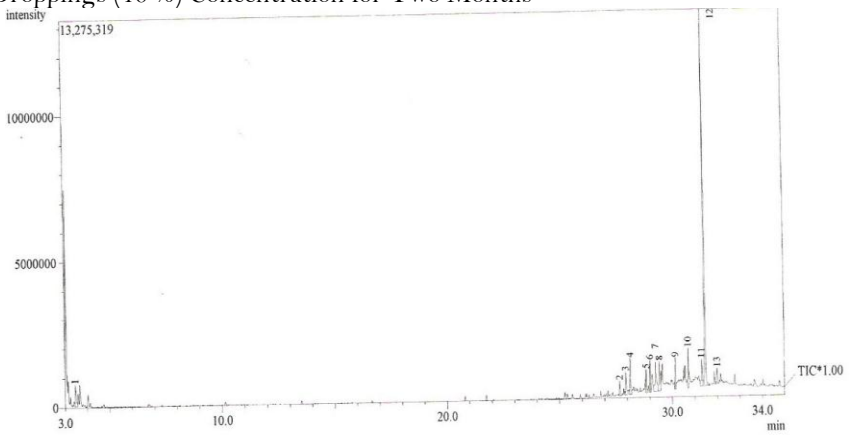


Figure-8. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with Chicken Droppings (20 %) Concentration for Two Months

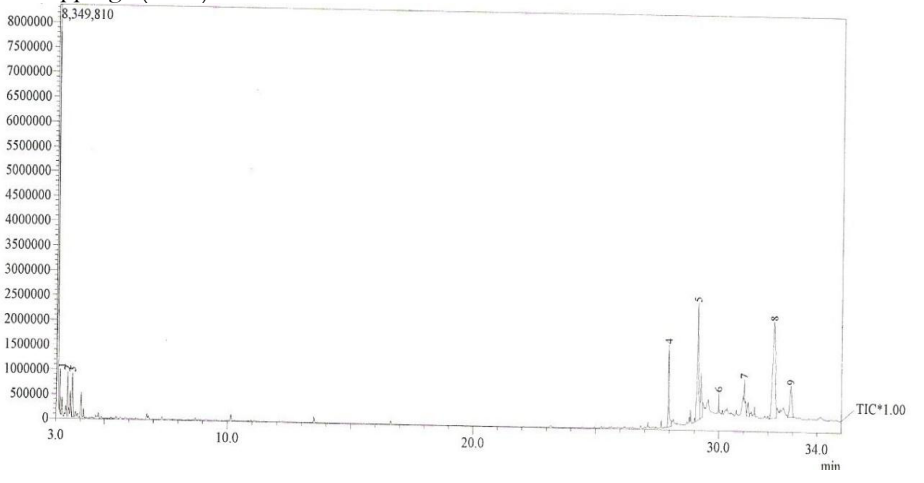


Figure-9. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with Chicken Droppings (30 %) Concentration for Two Months

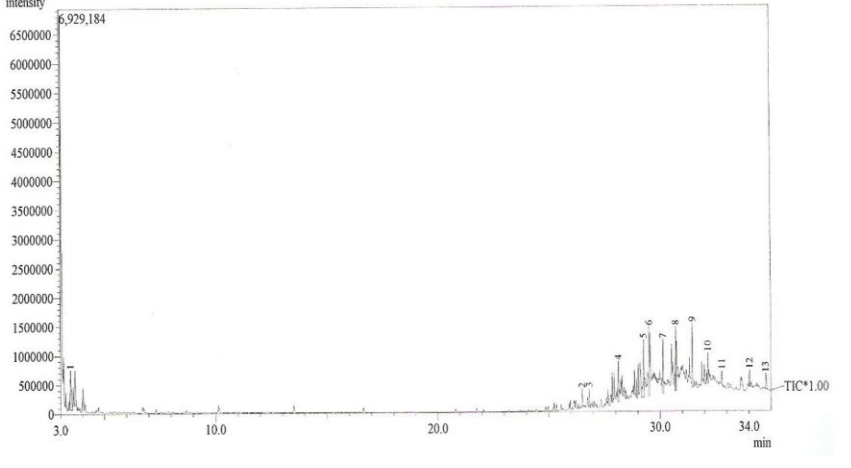


Figure-10. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with a Combination of Chicken Droppings and Cow Dung (10 %) Concentration for Two Months

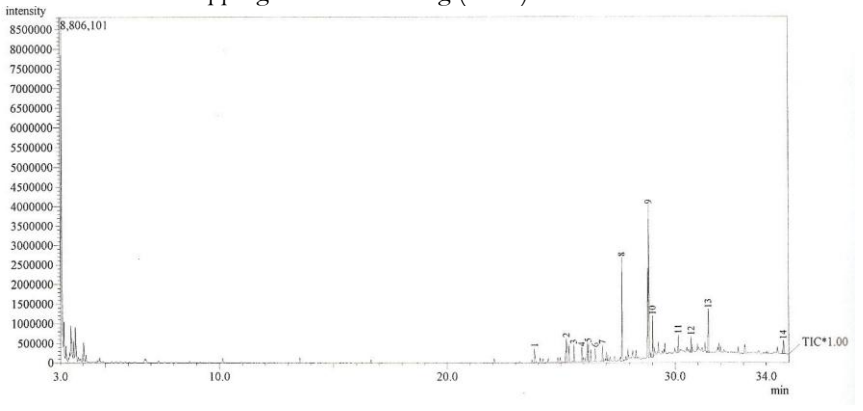


Figure-11. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with a Combination of Chicken Droppings and Cow Dung (20 %) Concentration for Two Months

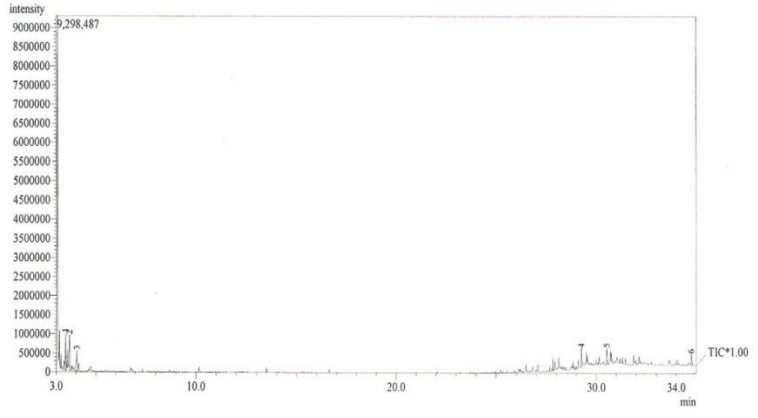
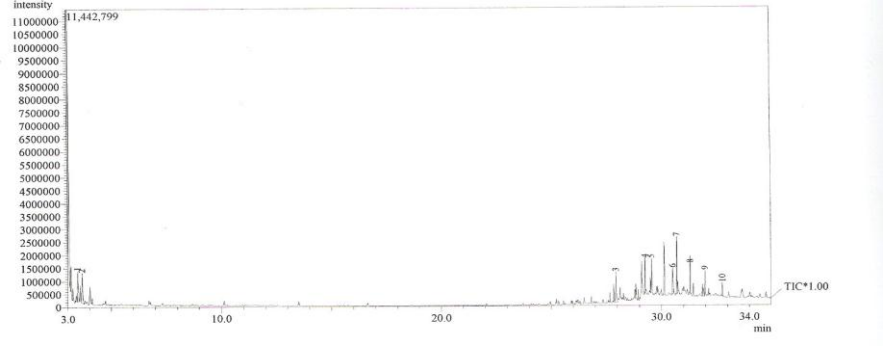


Figure-12. GC-MS of Palm Oil Mill Effluent Polluted Soil Extract after Bioremediation with a Combination of Chicken Droppings and Cow Dung (30 %) Concentration for Two Months



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