RECOVERY OF CRUDE OIL CONTAMINATED SOIL USING GROUNDNUT SHELLS AND DECOMPOSED MELON FRUITS

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Abstract

Candida has been reported has one of the most prevailing yeast genera. *Candida* is of diverse species that are commonly leave on soil and in the mucosal surfaces of genitourinary tract, gastrointestinal tract, and the mouth of human and have ability to cause oral thrush or vaginal thrush. The incidence of *Candida* infections has dramatically increased in recent years due to a significant increase in cases like HIV/AIDS, cancer, diabetes mellitus, long time use of antibiotic and tissue transplant. It is a cross-sectional study involving patients diagnosed of Candidiasis. The aim of this study is to identify, characterize and determine the prevalence of *Candida* species isolated from clinical specimens in selected health care facilities in Ilorin, Kwara state, Nigeria using conventional methods such as CHROM agar *Candida* differential media and Germ tube test. Of the 232 isolates recovered from Sabouraud dextrose agar, sub-cultured on CHROM agar and tested for Germ Tube Test. *C. albicans* 131(56.5%), has the highest prevalence, non-albicans such as *C. tropicalis* has the prevalence of 36(15.5%), *C. dubliniensis* 27(11.6*%), C. glabrat*a 14(6.0%), *C. krusei* 12(5.2%), there was mixed growth 7(3.0%) and 3(1.3%) did not grow on CHROM agar. *Candida albicans* is the most prevalence of *Candida* species isolate from clinical specimen in Ilorin, Nigeria in this study (56.5%) and *C. albicans* is also the most prevalence in Female (59.6%) while *C. tropicalis* has the highest prevalence in Male (36.8). Age group between 20 -29 has the highest prevalence (64.5%). Clinical specimens that produce highest number of *Candida* species in this study was High Vaginal Swab (58.2%) and pelvic inflammatory disease was the most clinical diagnosis (34.1%) and female subject has the highest prevalence of *Candida* infections (91.8%) while male has (8.2%) in Ilorin, Nigeria. There was a statistical significant difference ($P < 0.01$) between age and *Candida specie*s distribution among participant.

Keywords: Bioremediation, organic wastes, oil spills, soil Microorganism

1. Introduction

The increase in demand for crude oil as source of energy and a primary raw material for industries has led to an increase in its production, transportation and refining which in turn has resulted in gross pollution of the environment due to spillage [1]. Oil pollution results from oil- well blow out, seepage, deballasting operations, sale and use of petroleum products, pipeline overflow and breakage and storage tank spill. Addition of oil to the soil as a deliberate policy of waste disposal and discharge of oilfield wastewater also leads to contamination [2]. Oil spillages in terrestrial environment affect the physicochemical properties of the soil thus endangering the growth performance of the plants due to prevention of water and oxygen from reaching the plant, thereby resulting to either the suffocating or loss of plant viability. Besides, oil spills alter the microbiological status of the affected environment [3,4,5,6].

Conventional oil spill counter measures include various physical, chemical and biological methods. Although conventional methods, such as physical removal, often are the first response option, they rarely achieve complete cleanup of oil spills. Therefore, bioremediation is a promising technology, particularly as secondary treatment option for oil cleanup. Bioremediation involves the transformation and breakdown of complex organic molecules through biostimulation and bioaugementation into simpler substances such as fatty acids, carbon dioxide and water [7]. Bioremediation has several potential

advantages over conventional technologies, such as being less costly, less intrusive to the contaminated site, and more environmentally benign in terms of its end products [5,8].

There are two main approaches to oil spill bioremediation. These are bioaugmentation, in which known oil degrading bacteria are added to supplement the existing microbial population; and biostimulation in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth limiting co-substrates. Inorganic fertilizer, cow dung, chicken droppings, corn cobs, spent mushroom have been used as bioremediation materials. Ijah and Antai [5] reported the potential use of chicken-drop microorganisms for oil spill remediation and biostimulation of crude oil spilled soil with periwinkle shells [9]. Agbor et al. [10] reported the effectiveness of cassava peels and poultry droppings in enhancing the degradation of crude oil polluted soil in south eastern Nigeria. Idowu et al. [11] screened organic wastes for bioremediation of spent lubricating oil polluted soil and found that the organic wastes (cow dung, corn cob, chicken droppings, rice husk, and sorghum husk) caused more oil bioremediation in the soil (5-6 folds) than the unamended soil. The investigators attributed the enhancement to nutrients and microorganisms inherent in the organic wastes.

Treatment of oil contaminated soil is necessary to protect water supplies, human health and environmental quality [12], owing to the fact that plants derive nutrients for their living from soil, animals derive theirs from plants and human derive theirs from plants and animals living on soil. In Nigeria, most of the terrestrial ecosystem and shoreline in oil producing communities are important agricultural land under constant cultivation, but petroleum pollutant has made the land unsuitable for agriculture. For these reasons, there is the need to screen organic wastes such as decomposed melon fruits and groundnut shells that may help restore oil polluted soil for a better use. These organic wastes are abundant in the Nigerian environment, cheap and contain nutrients such as nitrogen and phosphorus needed by microorganisms to break down pollutants. The use of these organic wastes in oil spilled bioremediation in soil also helps to reduce waste disposal problem caused by these wastes. The aim of the study was to evaluate the potential of decomposed melon fruits and groundnut shells to restore oil spilled soil. The microorganisms associated with oil biodegradation in the amended soil were also identified.

2 Materials and Methods

2.1 Collection and Processing of Samples

Soil was collected from a non-oil polluted site in Bosso Campus of the Federal University of Technology, Minna, Nigeria, in polythene bags, and transported to the biological garden for use in bioremediation studies. The crude oil (Bonny light crude) was collected from Kaduna Refinery and Petrochemical Company (KRPC), Kaduna, Nigeria. The organic wastes, groundnut shells (GS) were collected from Kulo in Kuje Area Council of the Federal Capital Territory, Abuja, Nigeria and melon fruits (MF) were collected from a farm in Chanchaga, Niger State, Nigeria. The decomposed melon fruits (the pods had been removed) and groundnut shells were sun dried for three weeks, after which they were ground and stored in the laboratory for use in bioremediation studies. The microbiological and physicochemical properties of the soil, groundnut shells and decomposed depodded melon fruits used are presented in Table 1.

2.2 Experimental design and treatment

Randomized complete block design (RCBD) was used and the treatments were carried out in three replicates at the Biological Garden of the Federal University of Technology, Minna, Nigeria. One kilogram of soil was introduced into an experimental pot and treated with 1% (v/w) crude oil and amended with 2% (w/w) groundnut shells or decomposed melon fruits as well as a combination of the two organic wastes. Non-oil polluted soil without any amendment served as control. The experiments were exposed for three months in the natural environment throughout the period of this study. Temperature and the amount of rainfall in the area were recorded. The rainfall ranged from 46.6mm to 159.4mm while the temperature ranged from 23.08^0C to 31.84^0C.

2.3 Microbiological analysis of organic wastes and soil

Microorganisms were enumerated in soil, groundnut shells and decomposed melon fruits by spread inoculating 1ml of serially diluted sample in Nutrient agar (NA), oil agar (OA) and Sabouraud Dextrose Agar (SDA) for the enumeration of aerobic heterotrophic bacteria, crude oil utilizing bacteria and fungi respectively. The inoculated NA plates were incubated at 30^0C for 48 h while SDA and OA plates were incubated at room temperature (28 ± 2^0C) for 3-5 days. Colonies which appeared on the plates were counted and expressed as colony forming units per gram (cfu/g) of sample. Pure isolates of bacteria and fungi were obtained by repeated subculturing on fresh NA and SDA respectively.

2.4 Characterization and identification of microbial isolates

Bacterial isolates were characterized based on Gram staining and biochemical tests. The biochemical tests included production of indole, catalase, oxidase, coagulase, urease, starch hydrolysis, motility and nitrate reduction test, carbohydrate fermentation and methyl red-voges proskauer (MR-VP) test. The isolates were identified by comparing their characteristics with those of known taxa using the Scheme of Holt *et al*. [13]. Fungal isolates were characterized based on microscopic and macroscopic appearances which comprised pigmentation, color of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spore, presence of special structure such as foot cell, sporangiosphore, or conidiosphore and the characteristic of spore head. The identities of the fungal isolates were determined using the scheme of Nagamant *et al*. [14].

2.5 Bioremediation Studies

2.5.1 Determination of crude oil biodegeneration in soil by gravimetric analysis method

Crude oil biodegradation in the soil was determined using the gravimetric analysis method [15]. Exactly 10ml of diethyl ether was added to 5g of oil contaminated (or amended) soil contained in a sample bottle and the suspension was vigorously shaken to extract the crude oil. This was done repeatedly until the soil extract became colorless. The crude oil-solvent mixture was decanted into a preweighed Petri dish. The solvent (diethyl ether) was allowed to evaporate overnight and the new weight of Petri dish plus residual crude oil was determined. The amount of crude oil recovered was calculated using the formula

% crude oil biodegradation:

$$\frac{\text{weight of crude oil (control)} - \text{weight of crude oil (deg}}{\text{weight of crude oil (control)}}$$

2.5.2 Determination of pH of amended soil.

The pH of the amended soil was determined using pH meter (3035 Jeriway model). Five grams of soil sample was suspended in 25mL of distilled water and mixed properly. The pH meter was standardized at pH 7.0 using phosphate buffer solution after which the pH of the soil sample was determined by inserting the electrode of the pH meter into the partly settled suspension.

2.5.3 Gas chromatographic-mass spectroscopic analysis of degraded crude oil

The residual crude oil-solvent mixture was filtered through Whatman No 1 filter paper. The solvent was allowed to evaporate overnight. The extractable crude oil was recovered and analysed on capillary gas chromatograph [16]. The samples were injected by split injection with helium as carrier gas. The oven temperature was programmed as follows: 2 minutes at 45^0C followed by 5minutes at 280^0C. The major hydrocarbon compounds were identified on the basis of their retention time, percentage of abundance and by comparing them with those of analytical standards of library spectrum.

2.6 Statistical analysis

Data generated in the study were analyzed using Analysis of Variance (ANOVA) to establish significant differences between treatments. A computer package SPSS 15.0. 2006 Version was used for the analysis.

Results

3.1 Microbial counts and identity

The counts of aerobic heterotrophic bacteria in oil polluted soil amended with decomposed melon fruits (MF) and groundnut shells (GS) as shown in Fig. 1 revealed that, after 3 months the average counts were higher in unpolluted soil than in oil polluted soil and amended soils. However, statistical analysis of the data using analysis of variance (ANOVA) indicated that there were no significant differences ($p>0.05$) among the treatments. Similarly, counts of fungi were higher in the unpolluted soil than other treatments (Fig. 2). However, the counts were not significantly different ($p>0.05$). The trend in decreasing order of bacteria and fungi counts were unpolluted > oil polluted soil> GS > MF > GS+MF. When remediation was applied, the number of microorganisms increased which showed that the remediating materials were effective.

The mean counts of the oil utilizing bacteria (COUB) in oil polluted soil amended with MF and GS are shown in Fig. 3. The results revealed that the oil polluted soil amended with groundnut shells decreased as the time of remediation progressed The mean counts of the crude oil utilizing bacteria (CUOB) were not significantly different ($p>0.05$) among the treatments.

Table 1 Microbiological and physicochemical properties of organic wastes and soil used

Parameters	Decomposed Melon fruits	Groundnut shells	Soil
pH	6.70	6.90	6.80
Total nitrogen (%)	0.13	0.06	0.06
Available phosphorus (%)	0.50	0.20	9.80
Potassium (%)	0.14	0.32	0.80
Calcium (%)	0.37	0.28	0.31
Organic carbon (%)	15.68	28.31	0.95
Moisture (%)	1.0	4.0	24.0
Sand (%)	NA*	NA	67.68
Silt (%)	NA	NA	5.0
Clay (%)	NA	NA	28.32
Total aerobic heterotrophic bacteria	$5.5x10^6$ cfu/g	$9.6 x10^5$ cfu/g	$5.0 x 10^4$cfu/g
Fungi	$4.0x10^4$ cfu/g	$4.0x10^3$ cfu/g	$2.4 x 10^5$cfu/g
Crude oil utilizing bacteria	$3.8x10^2$cfu/g	$4.0x10^3$ cfu/g	$5.3x10^3$ cfu/g
Crude oil utilizing fungi	$1.6x10^2$cfu/g	$3.4x10^2$ cfu/g	$2.4x10^2$ cfu/g

*NA: Not applicable; cfu/g: colony forming units per gram.

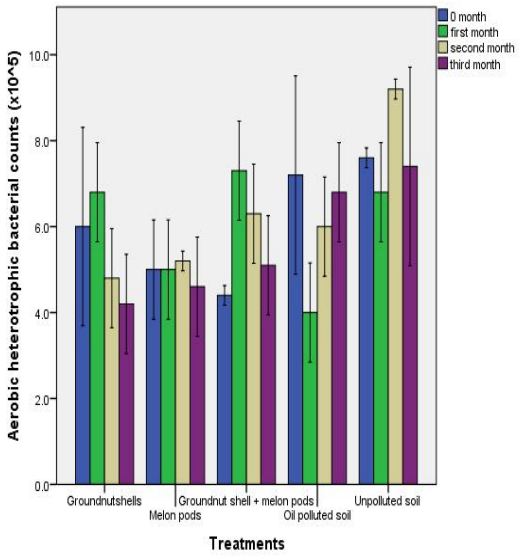

Fig. 1: Total aerobic heterotrophic bacterial counts in crude oil polluted soil (OPS) amended with: Groundnut shells (GS), decomposed fruits (MF), GS + MF, UPS; unpolluted soil (UPS)

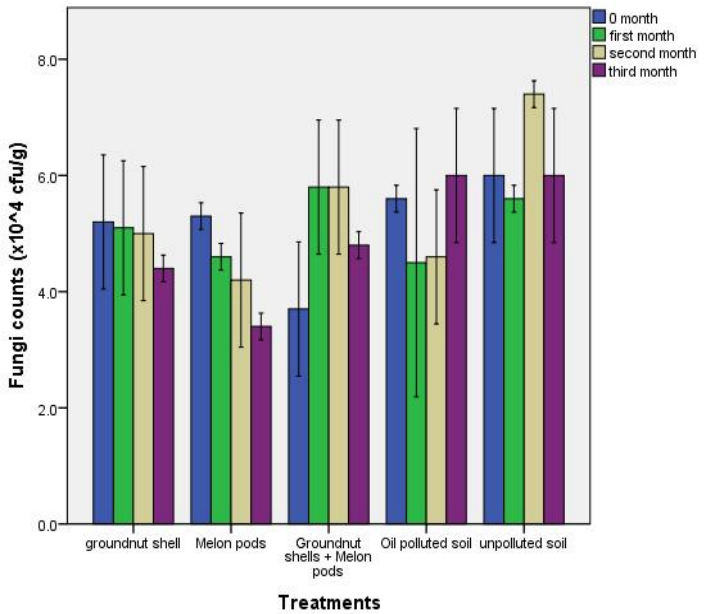

Fig. 2: Total fungal counts in crude oil polluted soil (OPS) amended with: Groundnut shell (GS), decomposed melon fruits (MF), GS + MF, unpolluted soil (UPS).

The counts of oil utilizing fungi in oil polluted soil amended with MF and GS were higher than the treatments, particularly one month after the bioremediation experiment was set-up (Fig. 4). The treatments with the exception of GF amended soil had less number of fungi after 2 months (Fig.4). There were significant differences ($p<0.05$) in the mean counts of the oil utilizing fungi between treatments. After 3 months, the counts in all treatments decreased extensively. However, oil polluted soil (OPS) had more fungal population among all treatments (Fig. 4).

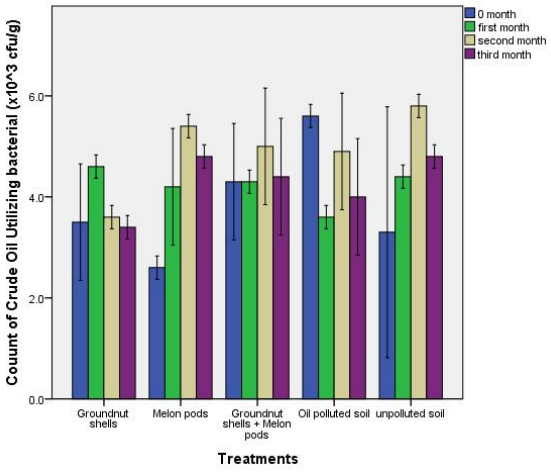

Fig. 3: Crude oil utilizing bacterial counts in crude oil polluted soil (OPS) amended with: Groundnut shells (GS), decomposed melon fruits (MF), GS + MP, Unpolluted soil (UPS)

Wide varieties of bacteria and fungi were isolated and identified from the amended, unamended and unpolluted soils. The Gram negative bacteria were identified as *Pseudomonas aeruginosa* (41.7%), *Klebsiella pneumoniae* (41.7%), *and E. coli* (16.6%). The Gram positive bacteria identified were *Bacillus subtilis, Staphylococcus auerus, Streptococcus mutans, Streptococcus faecalis*, and *Micrococcus* sp. *B. subtilis* was more consistently isolated and constituted 55.6% of the Gram positive bacterial isolates. The fungi were *Aspergillus niger, Asergillus fumigatus, Penicillium notatum, Aspergillus flavus, Mucor* sp. *a*nd *Fusarium* sp.

3.3 Crude Oil Bioremediation in Soil

3.3.1 Weight loss of crude in soil

The rates of remediation in unamended polluted soil were slow as compared to oil polluted soil amended with organic wastes (Table 2).

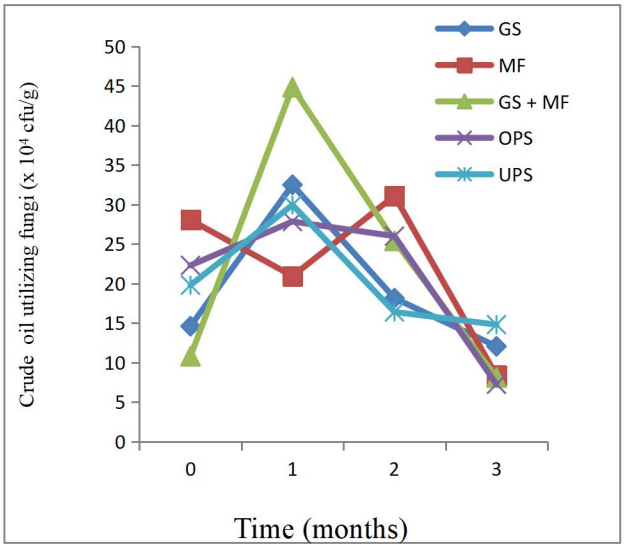

Fig. 4: Crude oil utilizing fungi counts (cfu/g) in crude oil polluted soil (OPS) amended with: Groundnut Shell (GS), decomposed melon fruits (MF), GS + MP unpolluted soil (UPS)

Amendment using GS and MF favored the bioremediation process from the start to the three months. It was observed that the rates of bioremediation caused by GS and MF individually were significantly different ($P<0.05$) from each other and higher than the unamended soil. After 3 months the results indicated that MF had the highest bioremediation potential among the treatments followed by GS+MF combination. In unamended soil the rate of bioremediation was relatively low after 4 months (Table 2).

3.3.2 pH of amended soil

The pH of crude oil polluted soil amended with groundnut shells (GS) ranged from 5.40 to 5.84 while that of soil amended with MF ranged between 7.02 and 8.0 (Fig. 5). The pH of crude oil polluted soil amended with a combination of the two substances had pH ranging from 6.0 to 7.8. pH of the polluted soil ranged between 6.41 and 6.66. Statistical analysis revealed that there were significant differences ($p<0.05$) among the pH of the amended, polluted and unpolluted soils after 3 months (Fig. 5).

3.4 GCMS profiles of residual crude oil extracts from amended soil.

The GC-MS indicated that Bonny light crude oil has various components (Fig.6). After 3 months of application of the oil to the soil, some

components of the oil were degraded (Fig.7). Three compounds (2, 6, 10-trimethylpentadecane, 2, 6, 10, 14-tetramethylpentadecane, and 2, 6, 10, 14-tetramethylhexadecane) were resistant to microbial attack in the oil polluted unamended soil (Fig 7). However, it was observed that new compounds such as 3,8-Dimethylpentadacane emerged in the treatment.

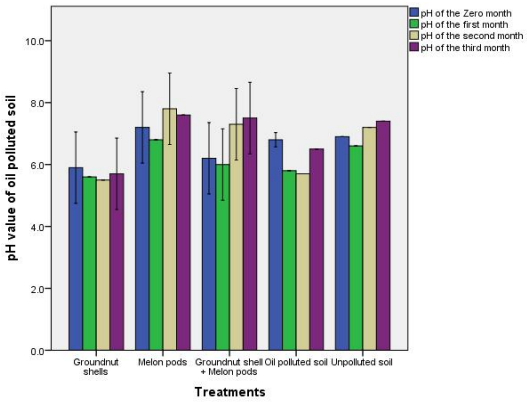

Figure 5: pH of crude oil polluted soil (OPS) amended with: Groundnut shells (GS), decomposed depodded melon fruits (MF), GS+MP, Unpolluted soil (UPS)

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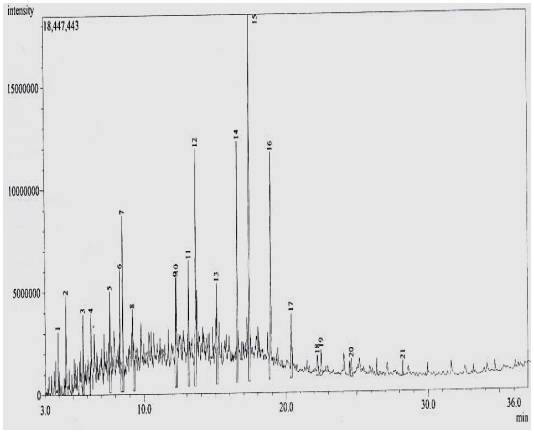

Fig.6: GC-analysis of undegraded Bonny light crude oil

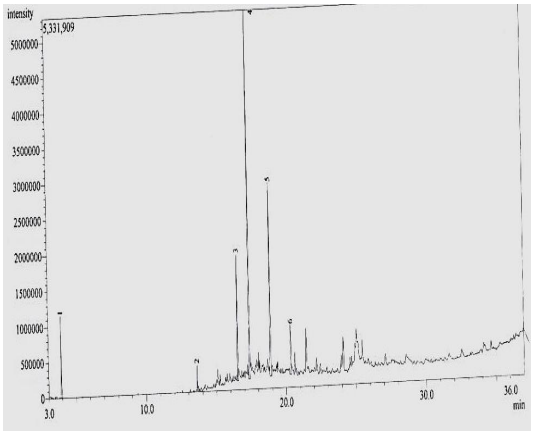

Fig. 7: GC-analysis of crude oil extract from unamended crude oil polluted soil after 3months

The GC-MS profile (Fig. 9) of residual crude oil extracted from oil polluted soil amended with decomposed melon fruits showed that certain components were degraded after 3 months which include 3,6-Dimethyl decane, 2,6,10,14-tetramethylpentadecane, 2,6,10-Tetrathyl pentadecane while 2,6,10,15-Tetrathylheptadecane and 2,6,10,14 - Tetrathylheptadecane were resistant to microbial attack. It was observed that some components which were not detected in the original oil sample emerged after 3 months. These include (9E)-9-1-consene (9-Eicosene), n-1-itexadecanol (Ado 154), 1-Docosene, Hystrene T70 and (9E) – 9-Haxadecenoic acid.

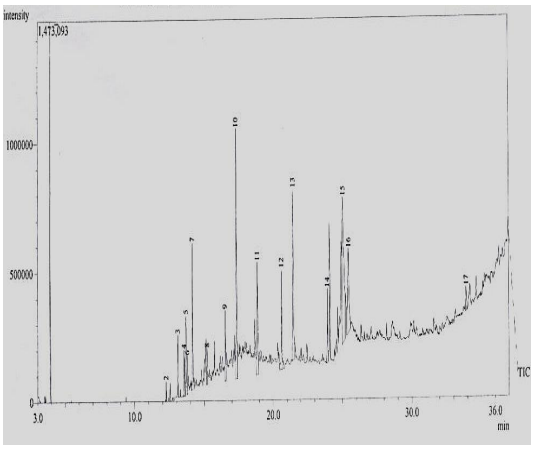

Figure 8: GC-analysis of crude oil extract from crude oil polluted soil amended with groundnut shells (GS) after 3months

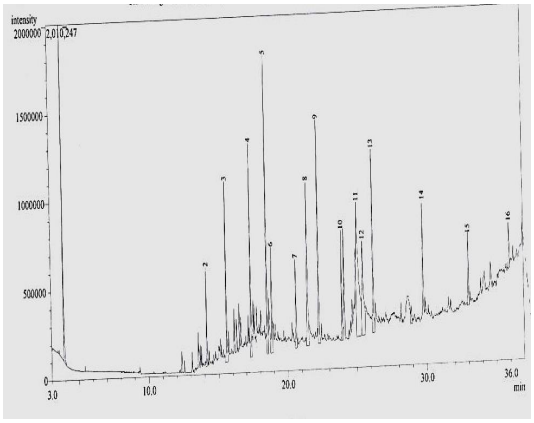

Fig. 9. GC-analysis of crude oil extract from crude oil polluted soil amended with decomposed depodded melon fruits (MF) after 3 months

The results of GC-MS analysis (Fig. 10) revealed that bioremediation had taken place in oil polluted soil amended with a combination of groundnut shells and decomposed melon fruits (MF) by activities of microorganisms contained in the organic wastes. This led to the biodegradation of certain oil components after 3 months which included 2,6,10,14-Tetramethylpentadecane and 11,12,-Dibromo-tetradecan-1-olacetate. However, some oil components emerged as a result of the biodegradation activities. These include Methyl-11,14-Icosadienonre, Pentadec-1-ene, Dimethyl nonane, Hexahydroaplotaxene, Hystrene –T-7. Methyl-10-octadeconate and methyl hexadecanoic acid could not be degraded after 3 months.

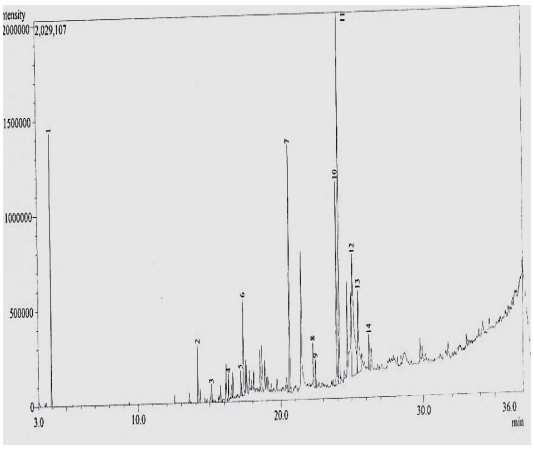

Fig. 11: GC-analysis of crude oil extract from crude oil polluted soil amended with groundnut shells (GS) and decomposed melon fruits (MF) after 3months.

4. Discussion

Decomposed melon fruits and groundnut shells contained phosphorous, nitrogen, and calcium. The percentage of microelements was higher in melon pods than groundnut shells. These nutrients are necessary for oil biodegradation [17,18]. Besides appreciable number of crude oil degrading microorganisms has been found in the organic wastes. The organisms were species of *Bacillus, Micrococcus, Staphylococcus, Klebsiella, Streptococcus, Aspergillus, Penicillium* and *Mucor.* These organisms have been reported as crude oil degraders by other investigators [19,18,20,21,8 and 29]

The microbial counts in the amended polluted soil decreased with increasing time of bioremediation. This could be as a result of depreciating value of organic wastes due to utilization by the oil degrading microorganisms [22]. The differences in the microbial counts from the various treatments could be as a result of the varying amounts of the microelements present. The counts in unamended polluted soil were consistent and unpolluted soils were persistently higher than the amended polluted soil which showed that their growths were not influenced by any growth factor. The amended polluted soil with groundnut shells showed higher counts within two months but depreciated in counts after 3 months. This could be as a result of low percentage of nitrogen, and available phosphorus which enhanced the capacity of oil degrading microorganisms found in the organic wastes. The findings of this study

are similar to the report of Abioye *et al.* [18]. One of the potential options for the control of oil spills in the soil is the application of inorganic fertilizers such as NPK and urea phosphate fertilizers to the oil spilled area because these compounds provide crucial nutrients (nitrogen and phosphorus) that encourage the growth and proliferation of the crude oil degrading microorganisms and accelerate oil biodegradation [23]. The rate of bioremediation of amended oil polluted soil with melon fruits was high after 3 months. This may be due to the fact that MF contain high amounts of nitrogen and available phosphorus which enhanced the efficient ability of the oil degrading microorganisms found in the organic waste [22]. The results of bioremediation revealed that there were significant differences ($p<0.05$) at each month among the treatments. Groundnut shells, though had shown an effect in bioremediation of the amended polluted soil, but not as the MF This could be due to the fact that the oil degraders found limited nutrients in organic wastes to enhance their ability in degrading crude oil in the amended soil [24].

The pH of the remediated soil revealed that there were significant differences ($p<0.05$) among the treatments. The pH of amended soil with GS was consistently within the range 5.40 -5.84 while that of soil amended with MF ranged between 7.02 and 8.00. This high pH value enhanced the ability of crude oil degraders. This is in agreement with the findings of some authors [25,11]. The GCMS analysis has revealed that Bonny light crude oil contains varying complex components of organic compounds. Some of these components of crude oil from unamended soil could not be found after 3 months of treatment with organic wastes, while some were resistant to microbial attack. The results revealed that oil components such as pentadecane were degraded after 3 months but compounds like (2, 6, 10-trimethyl pentadecane; 2, 6, 10, 14 - Tetramethyl Pentadecane and 2, 6, 10, 14 tetramethyl hexadecane resisted biodegradation. This result is similar to the finding of Yahaya and Bappa [26]. Some components were degraded after 3 months amendement of oil polluted soil with GS. This implies that those components of crude oil degraded by the microorganisms had been utilized as a source of carbon and energy during their metabolic processe [27]. The emergence of new components from Bonny light crude after 3 months of treatment, such as 1-Bromo-4-nonene; 1-4-exyl(-2-nitrocyclohexane; cresol-2,6-ditert-butyl (9E)-9-1-Consene (9-Eicosene); n-1-itexadecanol (Ado 154); 1-Docosene; Hystrene T70 and methyl (-11-14-icosadienonre, Hexahydroaplotaxene could be possible because the microorganisms contained in the organic wastes or present in the soil produced emulsifying agents which broke up the complex oil components into simpler components thereby increasing their abundance. These results are consistent with the findings of Alon and Amirav [16]. The disappearance of significant quantity of some of these components from the amended and unamended soil could also be related to abiotic factors, such as evaporation and photodegradation [27,28].

Conclusions

The organic wastes (groundnut shells GS, and decomposed melon fruits, MF) used in the present study contained nitrogen, phosphorus and crude oil degrading microorganisms necessary for bioremediation of crude oil polluted soil. The microorganisms played a major role in biodegradation of the crude oil pollutants. MF raised the pH of the oil polluted soil to alkaline level, reflecting its buffering capacity due to the high content of calcium. The GCMS analysis of the residual crude oil proved that bioremediation was enhanced by the organic wastes. It is important to avoid organic wastes with high amount of carbon compounds and low amounts of nitrogen and phosphorus since such wastes may not promote biodegradation of oil pollutants. MF are recommended for integrated oil spill control programs because of its high content of nitrogen, phosphorus, and calcium which encouraged crude oil degradation by microorganisms.

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