Bio-Degradation of Spent Oil in Contaminated Soil Using *CitrullusColocynthis* Peeling and Other Plant Wastes

ABSTRACT

Citrulluscolocynthis is a food and cash crop, planted mostly in the northern part of Nigeria and generates much waste after processing the seeds. These are disposed indiscriminately at the mills site and landfill, causing land pollution. At the southern part of Nigeria, oil spillage is a major source of land pollution. There is need to reclaim polluted lands for farming and other purposes. This study investigated the abilities of plant wastes, eg*Citrulluscolocynthis*peels, for the enhancement of the biodegradation of spent oil in contaminated soils. Contaminated soil in microcosms, A to J, were treated with plant wastes,duplicated to give 20 microcosms and incubated for 180 days. Periodic soil sampling from each microcosm was followed by cold extraction with dichloromethane and residual oil were analysed via GC/MS. The ANOVA showed that the stimulants accounted for 55.2 % of the total variation in the biodegradation result, with *P*=.000. The compounds identified ranged from C_{10}- C_{35}. The highest molecular weight compounds were 17-Pentatriacontene, 490.93 g/mol and 1-Hexacosene, 490.93 g/mol, while the lowest was p-Menth-8(10)-en-9-ol, cis, 154.25 g/mol. The compounds with the highest and lowest area percentage, 36.04 % and 0.97 % were 9-Octadecenamide, (Z)-, and 9,9-Dimethyl-9-sila-9,10-dihydrophenanthrene respectively. About, four alkanes, six alkene, fourteen oxygenated, five nitrogen-containing and eight aromatic compounds were identified after biodegradation. There was no significant difference between the performances of each of the stimulant in the microcosms. There was extreme significant difference between the performance of the stimulated microcosms and the natural attenuation containing NaN_3, with *P*= .000 at 0.05 significant level. The results show that the bio-stimulants have abilities to enhance the biodegradation of the spent oil by the inherent microorganisms better than the attenuated microcosms and pollutants from one part of the country can be used to treat pollutants in other parts.

KEYWORDS: *Citrulluscolocynthis, plant wastes, biodegradation, bio-stimulation, GC/MS, land/soil pollution*

INTRODUCTION

Soil pollution may be caused by agricultural pollutants such as fertilizer, pesticides, insecticides, weedicides; improper disposal of domestic, agricultural and industrial waste; detrimental soil management methods; radioactive materials, Oil spillage; etc [1]. In Niger Delta, Oil spillageis the major source of soil and water pollution, which is particularly of anthropogenic origin. The causes of oil spillage include:indiscriminate disposal of oil at the mechanic workshops, vandalized oil pipeline, crude oil exploration; accidental spillage at fillingstations, loading and pumping station; accidental spills during drilling from oil wells, spillage during refuelling and lubrication of trucks and trains, etc. [1,2]. The indiscriminate disposal of spent oil at the mechanic workshops is of great concern. Spent oil is the dark brown coloured waste engine oil generated from automobiles after servicing vehicles [3], and contain heavy metals [1,4-5] , mono cyclic and polycyclic aromatic hydrocarbon(PAHs) [6-7]. The toxic nature of spent oil makes it detrimental to plants, animal and man when it enters farmland, waterways and foodchain[3, 8]

In 2022, Nigeria produced about 59.06 % *CitrullusColocynthis* in the African continent, making her the largest producer of *CitrullusColocynthis* in Africa [9]. *CitrullusColocynthis*peels or husks are generated manually or mechanically in the processing of *CitrullusColocynthis* to obtain melon seed for cooking purposes [10]. In northern part of Nigeria, where *CitrullusColocynthis* farming is on the increase, the peels are disposed or burnt indiscriminately to cause soil and air pollutions, respectively [11]. Notwithstanding, *CitrullusColocynthis* peels contain proteins (15.5%), carbohydrates (69.59%), lipid (2.05%), fibre (6.07%) and ash (6.79%) [12]. Its minerals content (%) are: Na (0.41), K (0.71), P (0.22), Ca (0.1), Mg (0.39), Fe (22.17), Zn (18.68) and Cu (7.13)[13]. However, according to Ajayiand

Lateef,[13], the major anti-nutrients of *CitrullusColocynthis*peels include: HCN (16.28 mg/kg), lectin (55.66 HU/g), trypsin inhibitor (23.51 TIU/g) and amylase inhibitor (19.32 AIU/g). It also contained cellulose (71.8%), hemicellulose (23.0%) and lignin (0.29) [14].

Plant peelings and waste are agricultural waste which have limited usage because they contain complex molecules e.g. cellulose, hemicellulose and lignin, and anti-nutritional compounds such as cyanogenic glycosides, oxalates, phytates and trypsin [13]. These agro-wastes are disposed indiscriminately in the environment causing pollution. However, they are biologically non-toxic to soil organisms but contain nutrients that are useful in the amendment of soils that are deficient in nutrients. *Citrulluscolocynthis,* commonly known asbitter apple or bitter melon is a fruit crop that belongs to the family *Cucurbitaceae*[15]and is *al*so known as *Colocynthis vulgaris* (Schrad) or *Colocynthis officinalis* (Schrad)[15]. Biodegradation is the process, which involves microorganisms using contaminants as their source of nutrient, thereby, breaking down and transforming the contaminant into non-toxic compounds [16-17]. Several methods of bio-degradation have been used to biodegrade spent oil in contaminated soils such as pytoremediation[18- 19], bio-augmentation [2, 20- 24], bio-stimulations [22- 23], bio-pile [25- 26]. Therefore,this study investigates the use of *Citrulluscolocynthis* peelings, from the northern part of Nigeria, and other plant waste for the effective degradation of spent oil in contaminated soil.

MATERIALS AND METHODS

Soil and Plant Waste Sampling

Uncontaminated soil was collected from a farm free of crude or spent motor oil at Sheda Science and Technology Complex (SHESTCO) community in Sheda using a metal soil auger. The samples were stored in a dark coloured sterile polyethylene bag to prevent direct sunlight on them, labeled accordingly and transported immediately to the laboratory for further analysis.The cassava peelings was collected from the Cassava and Akpu market opposite National Mathematical Centre; *CitrullusColocynthis* peels were obtained at melon processing mill, Dabi; Sawdust was collected from the timber market Gwagwalada; fertilizer was purchased at Gwagwalada market.

Processing of Samples

The soil sample was sieved through a 2 mm pore size mesh to get rid of large debris. The plant wastes were air dried at 60 □C in the oven[27] until constant moisture content was obtained, pulverized, sieved using 2 mm sieve and stored in a labeled container. The drying of the cassava peels was too reduce the cyanide content that would endanger the inherent microorganisms[28].

Microcosm Setup

Soil samples of about 2 kg were weighed into each of about 10 plastic bucket and then contaminated with 125 ml of spent motor oil. These were allowed to stand for three days to allow the volatilization of toxic volatile compounds and for the soil to get used to the oil. Thereafter, the soil in the buckets were adequately mixed and allowed to stand undisturbed for two week. About 150 ml of distilled water was added every week to each bucket to increase the moisture content of the soils. It was mixed adequatelyto increased aeration.The plastic buckets were labelled A to J and treated with the follows bio-stimulants:A was treated with 400 g of NPK (15:15:15) fertilizer; B was treated with 400 g of cassava peeling; C was treated with 400 g *CitrullusColocynthis*peels; D was treated with 400 g Sawdust; E was treated with 200 g of *CitrullusColocynthis*peels and 200 g of cassava peelings; F was treated with 200 g of cassava peelings and 200 g of Sawdust; G was treated with 200 g of *CitrullusColocynthis* peels and 200 g of Sawdust; H was treated with 133.33 g of cassava peeling, 133.33 g of *CitrullusColocynthis peels* and 133.33 g of Sawdust; I contained only oil, no treatments (control); J containedauto-claved soil samples poisoned with 400 ml of sodium azide solution at 6.5%, as it inhibits the growth of microorganisms [29].

Sampling

The microcosm experiments were setup in duplicates, giving 20 experimental setups. Thorough mixing and addition of distilled water for adequate microbial growth was ensured. Sodium azide was added in Microcosm J, after 20, 40, 60, 80, 100 and 120 days of incubation of soil. Periodic sampling from each plastic buckets was carried out at 60^{th}, 120^{th} and 180 days to measure the residual oil after bio-degradation by the inherent microorganism following bio-stimulation with the different plant

wastes. Approximately 4 g of soil samples were from different portions of the plastic bucket to form a composite sample. This was done for all the 20 plastic buckets.

Extraction

The samples were analysed for the extent of bio-degradation of spent oil via cold extraction method.The residual oil in each sampled soil was cold extracted following the method of NnaOrji [6] using dichloromethane as solvent.
The oil extracts were analyzed using GC/MS analysis. Agilent technology 7890A GC system and Agilent technology 5975C ALMS were used for the analysis. Details of the GC parameters include: Stationary phase:Length, 30 m; Diameter, 0.32 mm; Thickness of Column, 0.25 µm; Oven Temperature, 60°C for 5 min; 60°C to 300°C at 10°C/min; Sample maximum Run Time, 30 min 50 sec. Detector Mass spectrophotometer: Detector Temperature 250°C, Injection Temperature 250°C, Volume of Injection: 1µl. The retention time and peak area percentage were recorded.

METHOD OF DATA ANALYSIS

Analysis of variance (ANOVA) was used to determine if the relationships between treatment conditions were statistically significant ($p < 0.05$) at various time points during the experiments. Tukey and Dunnett's multiple comparisons test at $\alpha = 0.05$ simultaneous confidence level were used for this analysis and results were generated using the Graph pad Prisms 7 Statistical Software ® Program

RESULT AND DISCUSSION

The result of the residual oil extracted from the pollution management of spent oil contaminated soil for 180 days is shown in Fig.1

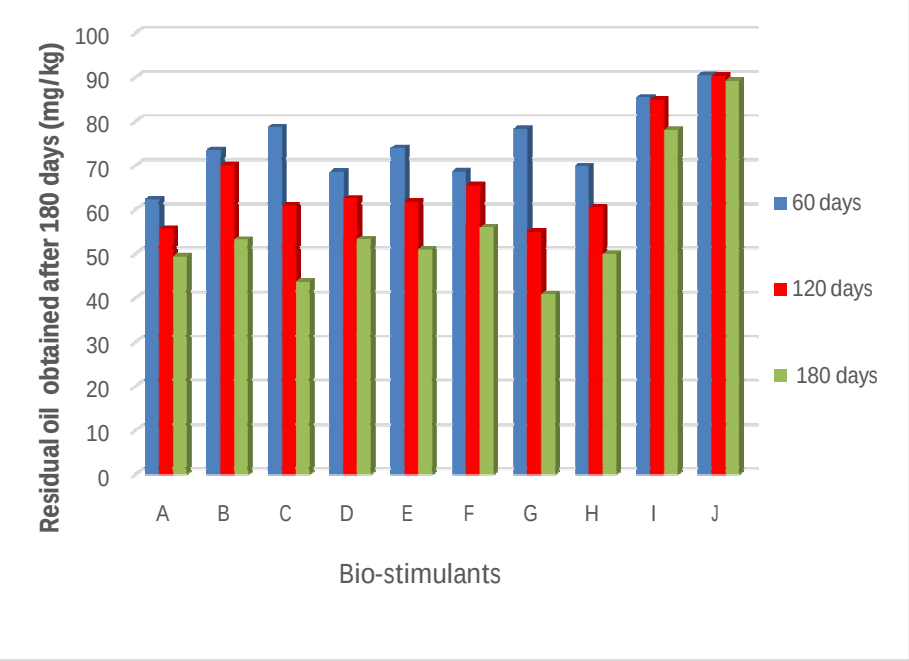


Fig 1: Residual oil (mg/ kg) extracted from spent oil artificially contaminated soil following bio-stimulation with plant waste

Throughout the 180 days of the study, the range of the oil extracted from the microcosms stimulated with the mixture of three organic fertilizers were from 49.95±8.20 to 69.88±6.53 mg/kg. The resultant synergy with two mixed stimulants, yielded residual oil that ranged from 40.88±4.21 to 78.3±0.09 mg/kg within the 180 days of study while the range of oil extracted from microcosms stimulated with

individual stimulants was from 43.7±5.27 to 78.66±1.87 mg/kg. However, within the 180 days of study, the residual oil from the microcosm stimulated with inorganic fertilizer ranged from 49.45±5.73 to 62.31±2.50. Meanwhile, the natural attenuation microcosms had residual oil that ranged from 78.03±1.45 to 90.54±0.14 mg/kg during the duration of the study. This shows that the natural attenuation microcosms had the highest residual oil after the 180 incubation days and indicates that the inherent microorganisms were not able to degrade much of the spent oil contaminant. This may be due to the poisoning effect of spent oil on the soil organisms [30], which led to the death of the soil organisms [31] and hence little or no bio-degradation took place.

From the ANOVA analysis, the interaction between the days of bio-stimulation and the stimulants accounted for 10.3 % of the total variation in the result obtained after extraction following bio-stimulation with a *P*=.005 showing a significant difference. Also, about 28 % variation in the results of the residual oil obtained was caused by the days allowed for the bio-stimulation of the inherent microbes while the stimulants used for the bio-stimulation accounted for 55.2 % of the total variation in the result, with *P*=.000. These effects were extremely significant, implying that for effective bio-degradation of oil contamination in the soil, bio-stimulant for stimulation of the inherent microorganisms must be available for the microbes to use the spent oil as a sole carbon source and thereafter biodegrade the oil contaminant [32] into non-toxic compounds.

Biodegradation following bio-stimulation after 60 days

Figure 2 shows the effectiveness of *CitrullusColocynthis* peels and other plant wastes used for the stimulation of the inherent microorganisms for the biodegradation of the spent oil in the contaminated soil during an incubation period of 60 days.

The residual oil extracted from bio-stimulated microcosms after 60 days ranged from 62.31±2.50 mg/kg NPK fertilizer to 78.66±1.87 mg/kg CitrullusColocynthis peels s while the residual oil from the control microcosms ranged from 85.4±6.05 to 90.54±0.14 mg/kg.

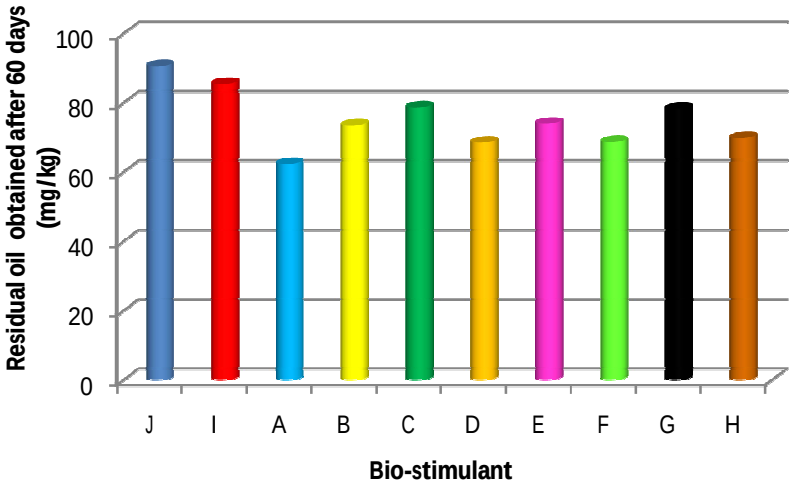


Fig 2: Residual oil (mg/ kg) extracted from waste oil artificially contaminated soil treated with plant waste after 60 days

The multiple comparisons test between the performance of the control with & without NaN_3 and each of the bio-stimulant in each monthly extraction following bio-stimulation with the plant wastes for the bio-degradation of the spent oil in the soil artificially contaminated with spent oil at $\alpha = 0.05$ was analysed. It depicts whether there is no significant difference between the performance of the bio-stimulated microcosms and the control without NaN_3 and that the control with NaN_3 or not. After 60 days of bio-stimulation, the multiple comparison tests revealed that the residual oil of62.31±2.50 mg/kg from NPK fertilizer; 68.50±7.35 mg/kg from Sawdust; 68.68±2.55 mg/kg from Cassava peelings + Sawdust and 69.88±6.53 mg/kg from Cassava Peeling + CitrullusColocynthis peels + Sawdust microcosms, were significantly lower than 90.54±0.1414 mg/kg extracted from control with NaN_3.

Their p-values of 0.0005, 0.0101, 0.0110, 0.0189 and 0.0062 respectively at 0.05 level of significance wereobtained.These performances of the bio-stimulated microcosms indicated the presences and viable activities of microorganisms in the microcosms amended with stimulants while the result from the control microcosms with NaN_3 shows little or no activities of microorganisms in the microcosms due to their poisoning cause by the NaN_3 added to the soil. In addition, the quantity of the residual spent oil in the bio-stimulated microcosms shows that biodegradation occurred via the inherent microorganisms that were stimulated by the availability of the nutrients from the stimulants.

Comparing the performance of the stimulants with that of the control without NaN_3after 60 days of bio-stimulation, the multiple comparison resultindicatesthat the residual oil of 68.50±7.35 mg/kg from the microcosms bio-stimulated with the inorganic NPK fertilizer significantly enhanced the bio-degradation of the spent oil in the contaminated better than the natural attenuation without NaN_3. The performances of the organic*CitrullusColocynthis* Peeling and the other plant wastes were not significantly different from the performance of the control without NaN_3 after the 60 days pollution management. The bio-stimulation of the inherent microbes with NPK fertilizer was the only stimulant that enhanced the bio-degradation of the spent oil better than the control without NaN_3. This implies that after the 60 days of bio-stimulation, *CitrullusColocynthis* peels and the other plant wastes used in amending the contaminated soil, performed exactly like the control without NaN_3, with no stimulant. This may be attributed to the time of approximately more than 60 days needed by the stimulants to incubate before the releasing their nitrogen and phosphorus [33] into the soil environment for adequate bio-stimulation of the inherent microorganisms and thereafter, enhance the bio-degradation of the spent oil in the contaminated soil. Hence, at the end of 60 days of bio-stimulation, all other stimulated microcosms except that with NPK performed just like natural attenuation microcosm.

Biodegradation following bio-stimulation after 120 days

The quantities of residual oil extracted from contaminated soil treated following bio-stimulated with plant waste after 120 days is shown in figure 3.

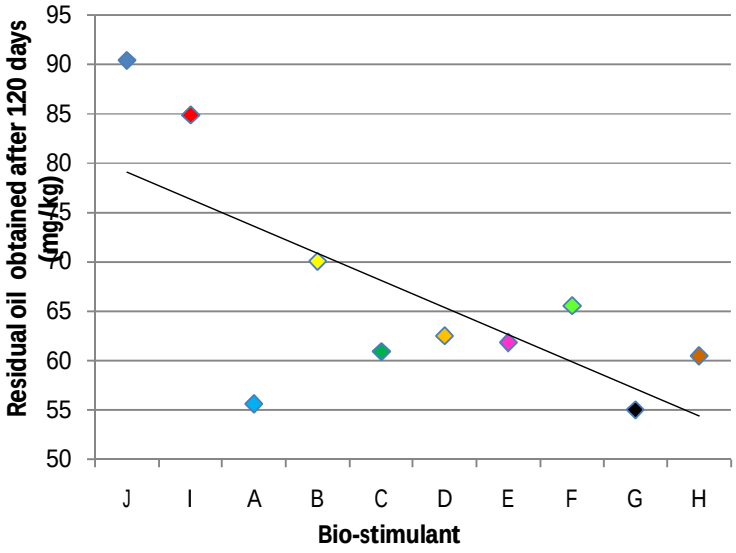


Fig 3: Residual oil (mg/ kg) extracted from waste oil artificially contaminated soil treated with plant waste after 120 days

The resultant residual oil extracted after 120 days from the bio-stimulated microcosms ranged from 54.99±6.91 mg/kg of CitrullusColocynthis peels s + Sawdust to 70.08±1.27 mg/kg of cassava peelings, whereas, the control experiments gave residual oil which ranged from 84.87±0.90 to 90.4±0.10 mg/kg.

After 120 days of bio-stimulation of the inherent microorganisms, the order of the quantities of the residual spent oil extracted from the amended microcosms was *CitrullusColocynthis* peels + Sawdust>NPK Fertilizer>Cassava peelings + *CitrullusColocynthis* peels + Sawdust>*CitrullusColocynthis* peels>*CitrullusColocynthis* peels + Cassava peeling>Sawdust> Cassava peelings + Sawdust> Cassava peeling. The least residual spent oil after 120 days of incubation was 54.985±6.9084 mg/kg from microcosms amended with *CitrullusColocynthis* peels + Sawdust while the highest residual spent oil, 70.08±1.2728 mg/kg, was from the microcosms with cassava peeling. However, the residual spent oil of 90.4±0.0990 mg/kg obtained from the control microcosms with NaN_3 was significantly the highest mean quantity of residual oil of all the microcosms after 120 days of incubation. Hence, the amendments significantly enhanced the bio-degradation of the spent oil contaminant in the contaminated soil better than the natural attenuation with NaN_3. The results showed the presence, viability and ability of inherent microorganisms to use up the nitrogen and phosphorus released by the stimulant for their bio-stimulation and eventually the degradation and reduction of the quantity of the spent oil in the contaminatedsoil after 120 days of incubation.

Also, the residual oil obtained from the control without NaN_3, after 120 days of incubation, was significantly higher than those from bio-stimulated microcosms with NPK fertilizer, *CitrullusColocynthis*, Sawdust, *CitrullusColocynthis* + Cassava peelings, *CitrullusColocynthis* + Sawdust and Cassava peelings + *CitrullusColocynthis* + Sawdust with p-values of 0.0003, 0.0041, 0.0086, 0.0063, 0.0344, 0.0002 and 0.0034 respectively at 0.05 level of significance. This shows that all the stimulant except cassava peelings were able to bio-stimulate the inherent microbe after an incubation period of 120 days. Thus, they have enhanced the bio-degradation of the spent oil, resulting in the reduction of the residual spent oil. It is possible that the residual cyanide content of the cassava peels affected the growth and bio-stimulation of the inherent microbes for the degradation of the spent oil [28].

Biodegradation following bio-stimulation after 180 days

Figure4 shows the performances of *CitrullusColocynthis* peels and other plant wastes used for the stimulation of the inherent microorganisms for the biodegradation of the spent oil in the contaminated soil during an incubation period of 180 days.

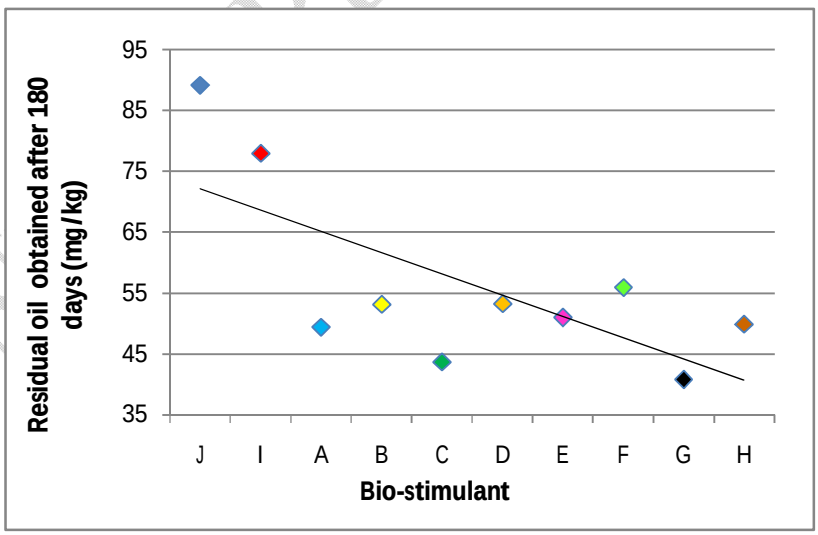


Fig 4: Residual oil (mg/ kg) extracted from waste oil artificially contaminated soil treated with plant waste after 180 days

The order of the extracted residual oil quantities from each of the plastic bucket microcosms after 180 days incubation period was G< C< A< H< E< B< D< F< J< I. At the end of 180 days of incubation, the lowest mean quantity of residual oil observed was, 40.88±4.21 mg/kg, extracted from *CitrullusColocynthis* peels + Sawdust, followed by a mean of 43.70±5.27mg/kg extracted from the

microcosms stimulated with only *CitrullusColocynthis* peels and thirdly by 49.45±5.73 mg/kg from NPK bio-stimulated microcosms. However, the highest mean quantity of residual oil, 55.98±3.50 mg/kg, was from microcosms containing cassava peelings + Sawdust. From the results, the microcosms containing *CitrullusColocynthis* peels were among the first four microcosms with the low mean quantities of residual oil after 180 days of incubation. After the duration of 180 days, the range of the extracted residual oil from the bio-stimulated microcosms was from 40.88±4.21 mg/kg of the *CitrullusColocynthis* peels + sawdust to 55.98±3.50 mg/kg whilst the range for the extracts from the control microcosms was from 78.03±1.45 to 89.2±0.07 mg/kg.

The quantities of spent oil extracted from the microcosms depicts the performances of the varying stimulants used to stimulate the inherent microbe before biodegradation. From the ANOVA analysis, there was no significant difference between the performances of each of the stimulant in the microcosms, implying that they similarly enhanced the bio-degradation of the spent motor oil by the inherent microorganisms. Hence, the microcosm containing only *CitrullusColocynthis* peels and those containing fractions of *CitrullusColocynthis* peels performed just like the other plant wastes use for the biodegradation of the spent oil in the different microcosms.In addition, there was extreme significant difference between the performance of the stimulated microcosms and the natural attenuation containing NaN_3, with p-values of <0.0001 and at 0.05 significant level. The indication is that, the plant waste in the bio-stimulated microcosms significantly enhanced the performance of the inherent microbes for the biodegradation of the spent oil in the contaminated soil, better that the natural attenuation microcosms with NaN_3, which yielded a residualoil of 89.2±0.07 mg/kg. Hence, the nitrogen components of the bio-stimulants released into the soil increased the activities of the microbes for the degradation of the spent oil in the soil better than in the controls without any bio-stimulant [34].

From Figure 4 and the ANOVA result after 180 days, there was no significant difference between the performance of the natural attenuation microcosms containing NaN_3 and that without NaN_3. The presence and effect of the sodium azide in the contaminated soil, which resulted in killing the microbe, reduced the soil enzyme activity and microbial population [35- 36], and did not really differ or made any difference compared to when the sodium azidewas not added. This proves that the inherent microbes were not actually active but dormant and incapacitated due to the presence of the spent motor oil in the soil. The contamination by spent oil caused harm, instability, discomfort to the soil organisms[37], and changes the physical, chemical and biological systems of the soil flora [38]. More so, the stirring to enable aeration and the addition of water to ensure maximal survival of the soil microbes every two week in the control without NaN_3 made no significant different or improvement to ensuring adequate growth of the inherent microorganisms.

From the ANOVA analysis, there was no significant difference in the performance of each of the stimulants with each other in reducing the residual oil in the contaminated soil. Hence, whether as singles or in synergy at between the 60, 120 and 180 days, the performance of all the stimulants were found to be the same. The overall implication is that all the stimulants performed equally alike without been statistically different from each other's performance in bio-stimulating the inherent microorganisms based on the residual oil obtained during the 180 days of bio-degradation process.

Monthly Performance of the Stimulant

The multiple comparisons test between the results obtain in each month with the rest of the months following bio-degradation enhancement by each stimulant for soil artificially contaminated with spent oil and bio-stimulated with plant waste at $\alpha = 0.05$ showed significant differences.

Microcosm A

The range of the residual oil extracted from the Microcosms A was 6.69 and 12.86 mg/kg after 120 and 180 days incubation period respectively. Theconventional NPK fertilizers release their nutrients faster and have high dissolution rates in the soil than the NPK slow releasing fertilizer that are coated [39- 40]. Thus, the convention NPK fertilizer used in this study dissolution quickly and released its nutrients faster within the 120 days of incubation period, which resulted to similar performances of the microbes in biodegrading the spent oil after 60 and 120 days without any significant different. Nutrient uptake by the inherent microbe was more within the last 60 days than during the first 60 days of incubation as shown in the ANOVA result. The quantity of oil extracted following bio-stimulation with convention NPK after 180 days was significantly lower that extracted afterincubation period of 60

days. Therefore, the ability of NPK (15:15:15) fertilizer to enhance the nutritional value of the soil for adequate bio-degradation after 180 days of bio-stimulation was found to be significantly higher than its performance after 60 days. The p value was 0.0256, indicating that time played a major role in the bio-stimulation by NPK, as an inorganic fertilizer.

Microcosm B

The mean residual oil extracted from cassava peel bio-stimulated microcosms were 73.47±7.02, 70.08±1.27 and 53.18±3.57 mg/kg extracted after 60, 120, and 180 days incubation period, respectively. From the result presented in Fig 1, there was no significant difference between the mean residual spent oil, 73.47±7.02 mg/kg, extracted from the microcosms bio-stimulated with cassava peels after 60 days incubation period and that, 70.08±1.27 mg/kg after 120 days. However, the mean residual oil, 73.47±7.02 mg/kg and 70.08±1.27 mg/kg, extracted after 60 and 120 days respectively, were significantly higher than 53.18±3.57 mg/kg extracted after 180 days. The enhancement of inherent microbes by Cassava peelings for the bio-degradation of the spent oil in the contaminated soil after 180 days was significant better after 60 and 120 days with p- value of 0.0006 and 0.0035 respectively. This could be attributed to the low level of nitrogen content of the peeling, which was consistent with that of and high level of cyanide content of cassava peelingwhich could be damaging to the inherent microbe [28, 40]. The result also shows that more than 60 days could be required for the surviving microbe to handle the cyanide, decay the peelings and use the released nutrient to make adequate contribution in the bio-stimulation of the inherent microbe. More so, it could be that the inherent microbes from cassava peelings took at least 60 days to get adapted to the spent oil contaminated soil before the use of the spent oil as sole carbon source for their survival.

Microcosm C

The results from Figure 2- 4 shows a gradual reduction in the quantity of residual spent oil extracted from the microcosms bio-stimulated with *CitrullusColocynthis*. The range of the residual oil extracted from this microcosm from 60 to 180 days incubation period was 34.96 mg/kg. The mean residual oil, 43.70±5.27 mg/kg, extracted from the microcosm after 180 days was significantly lower than the mean of 60.92±1.86 mg/kg extracted after 120 days of bio-stimulation with *CitrullusColocynthis.* In addition, the mean residual spent oil, 60.92±1.86 mg/kg, extracted after 120 days of incubation was significantly lower than 78.66±1.87 mg/kg extracted after 60 days of bio-stimulation. This means that the enhancement of the bio-degradation of the spent oil in the contaminated soil by *CitrullusColocynthis*husk*,* was significantly better after180 days than after 60 and 120 days of bio-stimulation at $p< 0.05$ and its performance after 120 days was significantly better than after 60 days. This implies that the nitrogen content of *CitrullusColocynthis*husk, released after the decomposition of *CitrullusColocynthis* husk, enabled the growth of the inherent microbes and increase the microbial activities for the biodegradation of the spent oil into non-toxic compounds [34]. Therefore, the bio-degradation of the oil contaminant with *CitrullusColocynthis*husk started from the beginning of the bio-stimulation after its decomposition and release of its nitrogen content for the survival and growth of the inherent microbe. Thus, *CitrullusColocynthis*husk made a significant improvement in the activities of the microbes towards reducing the oil contaminant from the beginning of the incubation period tothe last day incubation period.

Microcosm D

The result of the bio-stimulation of the inherent microorganisms for the biodegradation of spent oil using sawdust is on Fig 2-4. The mean residual oil of 68.50±7.35 mg/kg extracted after 60 days of bio-stimulated was significantly higher than 53.30±5.94 mg/kg extracted after 180 days incubation period. There was no significant difference between the quantities of residual oil extracted after 60 and 120 days and between 120 and 180 days of biodegradation. Hence, bio-stimulation of inherent microbes by the addition of sawdust made a significant enhancement in biodegradation, as there was a reduction in the quantity of residual oil after180 days better than after 60 days with p value of 0.0082 at 0.05 significant levels. This may be due to the long-time taken for the complex molecule of the sawdust to decay and let down its nutrient for uptake by and growth of the inherent microbe.

Microcosm E

The results of the periodic biodegradation of spent oil contaminated soil using CitrullusColocynthis peels + Cassava peelings in microcosm E, is displayed in Fig 2-4. The mean residual oil extracted at

the end of the 60, 120 and 180 days biodegradation following bio-stimulation with CitrullusColocynthis peels + Cassava peelings were 73.99±1.31, 61.80±2.69 and 50.98±1.59 mg/kg. From the results, there was a gradual reduction in the quantity of residual oil extracted from the microcosm during the incubation period. The gradual reduction of the extracted residual oil must have beendue to the enhancement of the nutrient for the inherent microbes for the bio-degradation of the spent oil in the contaminated soil with spent oil by *CitrullusColocynthis* peels + Cassava peelings. The synergy between *CitrullusColocynthis* peels + Cassava peelings in the biodegradation process after 120 and 180 daysbio-stimulation, yielded residual oil of 61.80±2.69 and 50.98±1.59 mg/kg which were significantly lower than that, 73.99±1.31 mg/kg, after 60 days with $p < 0.05$. This could be due to the time needed by the inherent microbes in the soil to handle and breakdown the cyanide content in *CitrullusColocynthis*peels [14] and cassava peelings [28, 40] before making any contributory bio-degradation of the spent oil, thereby reducing the residual oil in the soil. The result may also be due to the lower nitrogen content of cassava peels, 1.69 % (Jude *et al.,* 2022) as compared to that of *CitrullusColocynthis* peels 15.5 % [12]

Microcosm F

The result of the synergy in biodegradation between cassava and sawdustis shown in Fig 2-4. After 60, 120 and 180 days the residual oil extracted from microcosm F, which contains cassava and sawdust bio-stimulants, were 68.68±2.55, 65.57±1.04 and 55.98±3.50 mg/kg, respectively. There was no significant difference in the performance of the combined bio-stimulant on the biodegradation the spent oil contaminant after 60 and 120 days of incubation. In addition, there was no significant difference in the quantities of residual oil, 65.57±1.04 and 55.98±3.50 mg/kg, extracted after the 120 and 180 days of biodegradation, respectively. However, the residual oil of 68.68±2.55 mg/kg, extracted from microcosm F after 60 days of bio-degradation was significantly higher than 55.98±3.50 mg/kg extracted after 180 days incubation period. This implies that the combine interaction between cassava and sawdust made significant effect in the enhancement of the ability of the inherent microbe for the bio-degradation and hence, the reduction of the quantity of oil extracted from the microcosm after 180 days and was lower than 68.68±2.55 mg/kg after 60 days with p value of 0.0275. This performance in synergy between sawdust and cassava was lower than their performance when used singularly. May be there was antagonistic effect by the stimulants in microcosm F on the inherent microbes stimulants. More so, the acidic nature, cyanide and low nitrogen content of the cassava peelings may have caused a reduction in growth of the microbes[42], hence effected the quantity of spent oil extracted from during the process. The time need to decompose the matrix of the sawdust may have as well affected the letting-down of the nutrient from the sawdust for the enhancement of the growth of the microbes.

Microcosm G

The combined interaction between *CitrullusColocynthis* peels + Sawdustin the enhancement of the growth of the inherent microbe for the bio-degradation of the spent oil and reduction of the quantity of the residual oil extracted during the process, yielded 78.30±0.09, 54.99±6.91 and 40.88±4.21 mg/kg after 60, 120 and 180 days biodegradation, respectively. The mean residual oil, 78.30±0.09 mg/kg, extracted after 60 days was significantly higher than 54.99±6.91 mg/kg after 120 days of biodegradation, while 40.88±4.21 mg/kg extracted after 180 days incubation period was significantly lower than 54.99±6.91 mg/kg extracted after 120 days. More so, the mean residual oil, 40.88±4.21 mg/kg, extracted after 180 days was extremely lower than 78.30±0.09 mg/kg extracted after 60 days of biodegradation process. This synergy between *CitrullusColocynthis* peels + sawdust produced a progressive significant difference in the reduction of the residual oil extracted from the bio-stimulated microcosms. This could be due to the high nitrogen content of the*CitrullusColocynthis* peels, which must have increased the activities of the inherent microorganisms in the soil for the bio-degradation and eventual reduction in the quantity of the residual oil extracted every 60 days of bio-stimulation. The effectiveness of the synergy between *CitrullusColocynthis* peels + sawdust in the reduction of the residual oil was better than their performance in individual microcosms.

Microcosm H

The residual oil, 49.95±8.20 mg/kg, extracted after 180 days of bio-stimulation using CitrullusColocynthis peels + Cassava peelings+ sawdust in synergy was significantly lower than 69.88±6.54 mg/kg, extracted after 60 days with p value of 0.0007 at 0.05 significant level. However, there was no significant difference between 69.88±6.54 mg/kg extracted after 60 days and 60.49±2.15

mg/kg extracted after 120 days and between 60.49±2.15 of 120 days and 49.95±8.20 of 180 days. The significant performance of the three-plant waste in the reduction ofthe quantity of residual oil extracted between 60 – 180 days may be due to time required by the inherent microbe to decompose the complex molecule of *CitrullusColocynthis* and the sawdust, handle the residual cyanide in the *CitrullusColocynthis*peels andcassava peeling and absorb the let-down nutrient.

At the end of the 180 days of biodegradation following bio-stimulation with all the microcosms containing combined bio-stimulants, CitrullusColocynthis peels + sawdusthad the lowest mean extracted residual oil of 40.88±4.21 mg/kg followed by CitrullusColocynthis peels + Cassava peelings+ sawdust with 49.95±8.20 mg/kg and thirdly byCitrullusColocynthis peels + Cassava peelings with 50.98±1.59 mg/kg. The microcosms with the highest extracted mean residual oil among the combined bio-stimulant was CitrullusColocynthis peels + sawdust with 55.98±3.50 mg/kg. From the multiple comparison test carried out using ANOVA, there was no significant difference between the performance of each of the combined stimulants in the reduction of the residual spent oil after 60, 120 and 180 days of bio-degradation process. This indicates an overall similar performance and effectiveness intheir ability to reduce the amount of residual oil extracted every 60 days of incubation period.

GC/MS ANALYSIS OF RESIDUAL OIL

The GC/MS analysis of the spent oil from car obtained from the mechanic workshop identified 21 compounds (Orji 2019). From the results, about eleven aromatic compounds were identified, which include: one mono-aromatic, nine di-aromatic and one tri-aromatic compounds named phenanthrene, 2- methyl-. However, only one alkane, 7-Methyl-octadecane, was identified (Orji 2019). About three cyclo-alkene were identified: 1,7-Dimethyl-3-phenyltricyclo [4.1.0.0(2,7)]hept-3-ene; 1,5,6,7-Tetramethyl-3-phenylbicyclo[3.2.0]hepta-2,6-diene; [4.2.2] Propella-2,4,7,9-tetraene(Orji 2019).

Fig. 5. shows the chromatogram of the extract from microcosms G,which contains contaminated soil bio-stimulated with *CitrullusColocynthis* peelsand Sawdust.

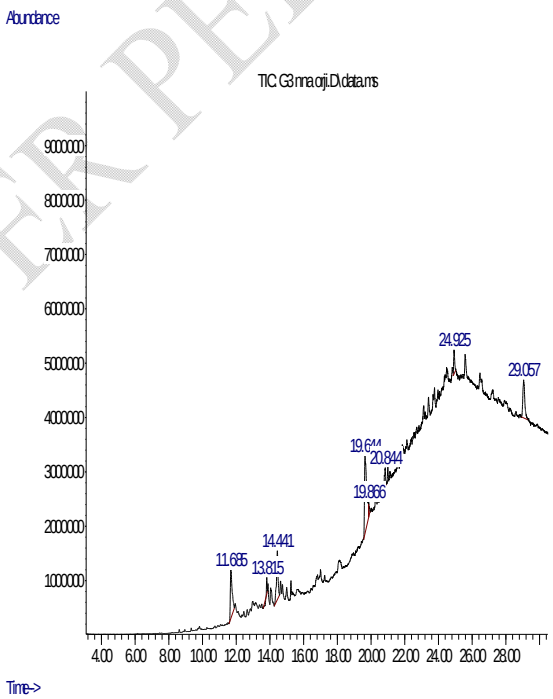


Fig 5. Chromatogram for residual oil extracted after bio-stimulation with yellow *CitrullusColocynthis* peels + Sawdust peelings for 180 days

This microcosm had a progressive reduction in the quantity of the residual oil among the stimulants combined in synergy. Table 1. reveals about eight compounds in the GC/MS analysis following 180 days of bio-stimulation with *CitrullusColocynthis*peels and Sawdust in the soils artificially contaminated with spent oil.

Table 1: Compounds found in spent-oil contaminated soil following bio-stimulation with *CitrullusColocynthis* peels and Sawdust for 180 days

S/NO	Compound Name	Molecular formula	Molecular weight (g/mol)	Area %
1.	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.48	36.04
2.	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	19.08
3.	2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile	$C_{15}H_{16}N_2$	224.30	16.35
4.	2-(3,4-Dimethoxyphenyl)-3-(5-Methoxy)phenyl propionic acid	$C_{18}H_{20}O_5$	316.35	16.05
5.	p-Menth-8(10)-en-9-ol, cis-	$C_{10}H_{18}O$	154.25	5.07
6.	Picolinyl 6,9-octadecadienoate	$C_{24}H_{37}NO_2$	371.56	4.71
7.	1-Tricosene	$C_{23}H_{46}$	322.61	1.72
8.	9,9-Dimethyl-9-sila-9,10-dihydrophenanthrene	$C_{15}H_{16}Si$	224.35	0.97

From the result, the compound found ranged from C_{10}- C_{24}. From table. 1, Picolinyl 6,9-octadecadienoate (371.56 g/mol) had the highest molecular weight followed by 1-Tricosene (322.61 g/mol) and thirdly by 2-(3,4-Dimethoxyphenyl)-3-(5-Methoxy)phenyl propionic acid (316.35 g/mol) while the compound with the lowest molecular weight was p-Menth-8(10)-en-9-ol, cis- (154.25 g/mol). About 62.5 % of the compounds identified in extract from G were oxygenated compounds while 37.5 % of the compounds contained nitrogen while 12.5 % were alkene. No alkane was identified. The other compounds include: one straight chain alkene, 1-Tricosene; an Alkenol compound, p-Menth-8(10)-en-9-ol, cis-; threenitrogen-containing compounds*:* 9-Octadecenamide, (Z)-, 2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile and Picolinyl 6,9-octadecadienoate; a silicon compound, 9,9-Dimethyl-9-sila-9,10-dihydrophenanthrene.

About fiveOxygen-containing compoundswere identified in the extracted residual oil as shown in Fig 6.

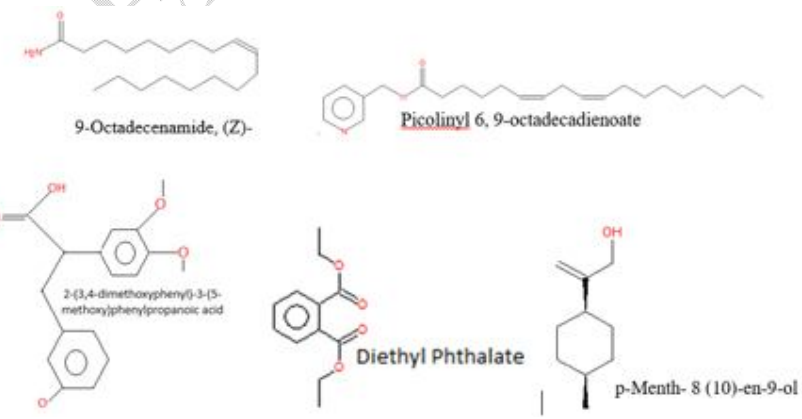


Fig 6, Oxygenated compounds identified in the residual extracts from Microcosm G

These compounds ranges C_{10}- C_{24}. The order of their molecular weight is: Picolinyl 6,9-octadecadienoate, $C_{24}H_{37}NO_2$>2-(3,4-Dimethoxyphenyl)-3-(5-Methoxy)phenylpropionic acid, $C_{18}H_{20}O_5$>9-Octadecenamide, (Z)-, $C_{18}H_{35}NO$>Diethyl Phthalate, $C_{12}H_{14}O_4$>p-Menth-8(10)-en-9-ol,

cis-, $C_{10}H_{18}O$. From the results, the five compounds containing oxygen had the following order in their area percentage indicating their concentrations in the extracted residual oil: 9-Octadecenamide, (Z)-> Diethyl Phthalate> 2-(3,4-Dimethoxyphenyl)-3-(5-Methoxy)phenylpropionic acid>p-Menth-8(10)-en-9-ol, cis- >Picolinyl 6,9-octadecadienoate.
The Aromatic compounds identified are shown in Fig 7. The 2 di-aromatic identified were 9,9-Dimethyl-9-sila-9,10-dihydrophenanthrene and 2-(3,4-Dimethoxyphenyl)-3-(5-Methoxy)phenylpropionic acid while 2 mono-aromatic compound identified were Diethyl Phthalate and Picolinyl 6,9-octadecadienoate

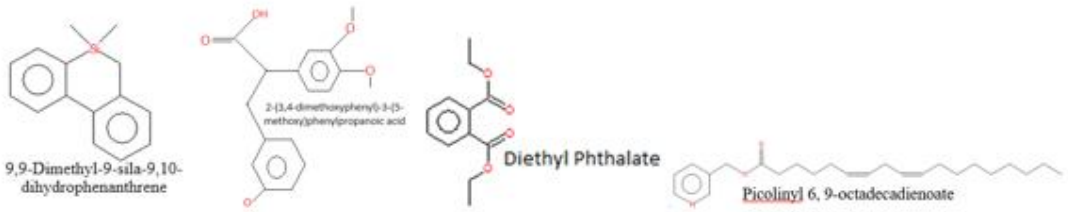


Fig 7, Oxygenated compounds identified in the residual extracts from Microcosm G

From table 1, Diethyl Phthalate had the highest area percentage, 19.08%, among the aromatic compounds, followed by 2-(3,4-Dimethoxyphenyl)-3-(5-Methoxy)phenylpropionic acid, with 16.05 % and thirdly by Picolinyl 6,9-octadecadienoate with 4.71 % while 9,9-Dimethyl-9-sila-9,10-dihydrophenanthrene, had the least area percent of 0.97 %.Diethyl phthalates, in the environment can be absorbed via ingestion, inhalation, and dermal absorption and its metabolites were seen in urine [41], serum [43], breast milk [44], etc. These metabolites have been linked to type II diabetes, overweight/obesity, asthma, poor semen quality in men and increased pregnancy loss in women[45].

The peak area percent of the compounds identified ranged from 36.04 to 0.97 %. 9-Octadecenamide, (Z)-Had the highest peak area while 9,9-Dimethyl-9-sila-9,10-dihydrophenanthrene had the least. Diethyl Phthalate and 2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile had the second and third highest peak area percentage, respectively.

The chromatogram of the residual oil extracted from microcosm C following bio-stimulation with *CitrullusColocynthis* peels after 180 days is shown in figure 8.

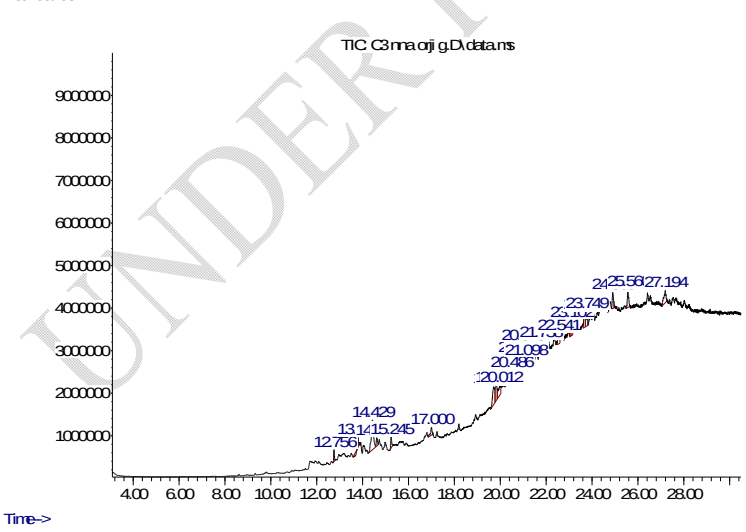


Fig. 8 Chromatogram for residual oil extracted after bio-stimulation with CitrullusColocynthis peels

After 180 days bio-degradation of spent oil in contaminated soil using *CitrullusColocynthis* peels, about 20 compounds were identified via the GC/MS analysis as shown in Table 2.

Table 2.Compounds found in spent-oil contaminated soil following bio-stimulation with *CitrullusColocynthis* peels for 180 days

S/NO	Compound Name	Molecular formula	Molecular weight (g/mol)	Area %
1.	2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile	$C_{15}H_{16}N_2$	224.30	15.42
2.	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296.53	8.61
3.	4-[3-Ethoxypropylamino]benzo-1,2,3-triazine	$C_{12}H_{16}N_4O$	232.00	8.04
4.	1-Bromo-11-iodoundecane	$C_{11}H_{22}BrI$	361.10	6.03
5.	2,4-Bis(dimethylbenzyl)-6-t-butylphenol	$C_{28}H_{34}O$	386.57	5.98
6.	17-Pentatriacontene	$C_{35}H_{70}$	490.93	5.90
7.	Cyclotetradecane,1,7,11-trimethyl-4-(1-methylethyl)-	$C_{20}H_{40}$	280.53	5.63
8.	1-Tricosene	$C_{23}H_{46}$	322.61	5.02
9.	1-Hexacosene	$C_{35}H_{70}$	490.93	4.72
10.	Z,Z-6,24-Tritriacontadien-2-one	$C_{33}H_{62}O$	474.00	4.45
11.	Cyclotriacontane	$C_{30}H_{60}$	420.80	4.30
12.	Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5.alpha.,6.beta.,12.beta.)-	$C_{28}H_{48}O_4$	448.68	4.08
13.	2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	$C_{25}H_{48}$	348.65	4.00
14.	1-Dodecanol, 2-octyl-	$C_{20}H_{42}O$	298.55	3.89
15.	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.00	2.98
16.	1,4-Diphenyl-1,3-butadiene	$C_{16}H_{14}$	206.28	2.64
17.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	2.24
18.	1,5,6,7-Tetramethyl-3-phenylbicyclo[3.2.0]hepta-2,6-diene	$C_{17}H_{20}$	224.34	2.19
19.	Pentadecane, 2,6,10,14-tetramethyl	$C_{19}H_{40}$	268.52	2.04
20.	Tetracosyltrifluoroacetate	$C_{26}H_{49}F_3O_2$	450.00	1.47

The peak area of the compounds ranged from 15.42 to 1.47 %. From the table 2, 2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile (15.42 %), Octadecane, 1-(ethenyloxy)- (8.61 %) and 4-[3-Ethoxypropylamino]benzo-1,2,3-triazine (8.04 %) were present in high amount while Tetracosyltrifluoroacetate(1.47 %) occurred in very low amount. The results show that among the 20 compounds identified, 5 % of the compounds had peak areas each within the range of 15.00-15.99%, 6.00-6.99 %, 3.00-3.99 % and 1.00-1.99%, respectively. Only 10 % of the compounds had area percentage within the range of 8.00- 8.99 % while 20 % of the compound had peak areas within 5.00-5.99 %. About 25 % of the compounds had peak areas ranging from 4.00-4.99 and 2.00-2.99 %, respectively.

From microcosm C, the compounds identified ranged from C_{11}- C_{35}. The molecules with the highest molecular weight in microcosm C, were 17-Pentatriacontene (490.93 g/mol) and 1-Hexacosene (490.93 g/mol), followed by Z,Z-6,24-Tritriacontadien-2-one (474 g/mol), and thirdly by Tetracosyltrifluoroacetate (450 g/mol), while the compound with the lowest molecular weight was 1,4-Diphenyl-1,3-butadiene, (206.28 g/mol). About 45 % of the compounds identified in table 2, were oxygen-containing compounds; 10 % of them contain nitrogen, 10 % contain halogen atoms, 25 % were alkanes and 25 % were alkenes.

The identified compounds include: 4 Alkane compounds:Cyclotriacontane, Cyclotetradecane,1,7,11-trimethyl-4-(1-methylethyl)-, 2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene)pentadecane, Pentadecane, 2,6,10,14-tetramethyland 1-Bromo-11-iodoundecane a halogenated alkane; 5 Alkene compounds: 17-Pentatriacontene; 1-Tricosene; 1-Hexacosene; 1,4-Diphenyl-1,3-butadiene and

1,5,6,7-Tetramethyl-3-phenylbicyclo[3.2.0]hepta-2,6-diene. In addition, 2 Nitrogen containing compounds were identified*:* 2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrileand 4-[3-Ethoxypropylamino]benzo-1,2,3-triazine. More so, Twohalogenated compounds named Tetracosyltrifluoroacetate and 1-Bromo-11-iodoundecane were found.

The aromatic compounds found after bio-stimulation with *CitrullusColocynthis* peelsare shown in Fig 9.

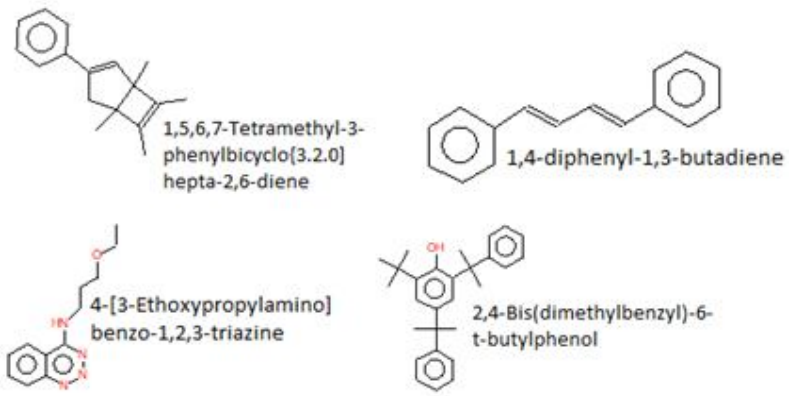
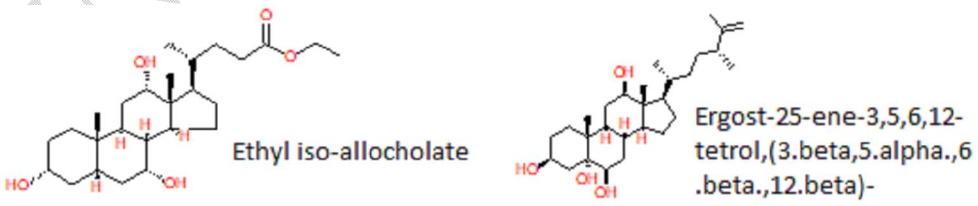


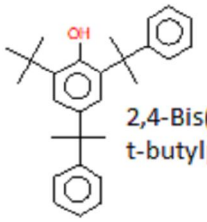
Fig 9. Oxygenated compounds identified in the residual extracts from Microcosm G

The only mono-aromatic and tri-aromatic compounds identified were1,5,6,7-Tetramethyl-3-phenylbicyclo[3.2.0]hepta-2,6-diene and 2,4-Bis(dimethylbenzyl)-6-t-butylphenol, respectively, while 1,4-Diphenyl-1,3-butadiene and 4-[3-Ethoxypropylamino]benzo-1,2,3-triazine were di-aromatic compounds. The order of their area percent was 4-[3-Ethoxypropylamino]benzo-1,2,3-triazine, 8.04 %>2,4-Bis(dimethylbenzyl)-6-t-butylphenol, 5.98 % >1,4-Diphenyl-1,3-butadiene, 2.64 % >1,5,6,7-Tetramethyl-3-phenylbicyclo[3.2.0]hepta-2,6-diene, 2.19 %.

From the GC/MS analysis results of the residual oil from microcosm bio-stimulated with *CitrullusColocynthis* peels, C, and *CitrullusColocynthis* peels& sawdust, G, about 20 % and 50 % of the compounds were aromatic compounds, respectively. However, according to Orji (2019) about 85 .71 % of the compounds identified in the spent oil from a car were aromatic compounds. The results show a reduction in the percentage of aromatic compounds identified after bio-degradation. Notwithstanding, the presence of these poly-aromatic compounds in the soil has the potentials of reducing the rate of biodegradation as they can poison, suffocate and lead to the death of the inherent microbes (Orji, 2019). In aquatic environment, they can cause health hazards to aquatic organisms and may bio-accumulate and lead to health risk in the body of man when these aquatic animals are eaten.

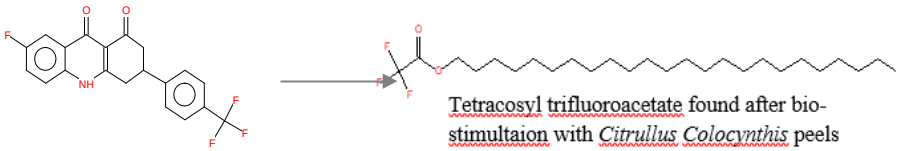
Some compounds identified after bio-stimulation with *CitrullusColocynthis* peels were different from those compounds identified in spent oil, indicating that they were inherent in *CitrullusColocynthis* peels. These compounds include:





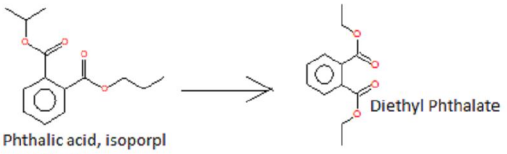
2,4-Bis(dimethylbenzyl)-6-t-butylphenol

The following related compounds were found before and after bio-degradation following bio-stimulation with plant waste:



Tetracosyl trifluoroacetate found after bio-stimultaion with *Citrullus Colocynthis* peels

7-Fluoro-3,4-dihydro-3-[4-[trifluoromethyl]phenyl]-1,9(2H,10H)-acridinedione found before bio-stimulation



Phthalic acid, isoporpl propyl ester

Phthalic acid, isoporpl propyl ester was found in spent oil before bio-stimulation while Bis(2-ethylhexyl) phthalate was found after bio-stimulation of the spent oil contaminated soil with *CitrullusColocynthis* peels and Sawdust.

2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile which was found in spent oil before bio-stimulation, with area percent of 16.70; after bio- stimulation with *CitrullusColocynthis* peels+ Sawdust; having 16.35%; with only melon, 15.42 % after 180 days of stimulation. It is a common Volatile Organic Compound that causes cancer in the colon, breast, prostate, etc [46].The 9-octanamideidentified after biodegradation could corrode and irritate the skin, cause allergic skin reactions; irritate and damage the eye, irritate the respiratory tract, cause organ toxicity and may cause long lasting harmful effects to aquatic life[44].

CONCLUSION

In conclusion, the bio-degradation of spent and crude oil in contaminated soils were effective following bio-stimulation with *CitrullusColocynthis* peels and other plant waste. The bio-degradation depended on time allowed for biodegradation and the ability of these waste to release nutrient, which enhanced the reduction of the extracted residual oil following periodic biodegradation by the inherent microorganisms. From the study, all the microcosms containing the leguminous wastes, *CitrullusColocynthis peels*, reduced the quantities of the oil extracted from the bio-stimulated soil. Therefore, *CitrullusColocynthis* peels and other plant waste investigated can be used to bio-degrade spent oil in contaminated soils so as to reclaim lands for farming purposes.

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