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Biodegradation of Spent Oil in Soil Using *Citrullus Colocynthis* Peels and Other Plant Wastes

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Spent oil, which contains hazardous substances that pose health risk to man and his environment, are disposed indiscriminately on land in most mechanic workshops. The waste oil find its way into farmland and water bodies causing contamination of water, plants and lands. The ingestion of contaminated water, plants and animals lead to serious health issues. The abilities of plant wastes such as *Citrullus colocynthis* peels were investigated for the enhancement of the biodegradation of spent oil in 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 Gas Chromatography/ Mass Spectrometry techniques (GC/MS). The results showed that the biostimulants accounted for 55.2 % of the total variation in the biodegradation result, $P<0.001$ at $\alpha = 0.05$. 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

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nitrogen containing- and eight aromatic- compounds were identified after biodegradation. There was no statistical significant difference between the performances of each of the stimulant in the microcosms, $P>0.1$ at $\alpha = 0.05$. There was an extremely significant difference between the performance of the stimulated microcosms and the natural attenuation containing NaN_3, with $P<0.001$ at 0.05 significant level. The results show that plant wastes have abilities to enhance the biodegradation of the spent oil. Hence, *Citrullus colocynthis* peels generated in the Northern Nigeria and other plant waste can be used for cost-effective and safe degradation of spent and crude oil in the Southern Nigeria for the reclamations of lands for agricultural purposes.

Keywords: Biodegradation; biostimulation; Citrullus colocynthis; GC/MS; land/soil pollution; plant wastes; spent oil

1. INTRODUCTION

Soil pollution may be caused by agricultural chemicals 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 spillage is 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 filling stations, loading and pumping station; accidental spills during drilling from oil wells, spillage during refuelling and lubrication of trucks and trains, etc. [1-5]. 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 [6], and contain heavy metals [1,7,8], mono cyclic and polycyclic aromatic hydrocarbon (PAHs) [9,10]. The toxic nature of spent oil are detrimental to plants, animal and man when it enters farmland, waterways and food chain [3,11].

Biodegradation is vastly accepted, as a safe, cost effective and eco-friendly method for the degradation of contaminants in the environment [12]. In this process, microorganisms use the contaminants as their source of nutrient and thereby, breaking down and transforming the contaminant into non-toxic compounds at the end of the process [13,14]. Several methods of biodegradation have been used to biodegrade spent oil in contaminated soils such as phytoremediation [15,16], bio-augmentation [2,17-21], biostimulation [9,19,20], bio-pile [22,23].

Most agricultural waste such as plant peels/waste, which are disposed indiscriminately in the environment causing pollution, are biologically non-toxic to soil organisms but contain nutrients that are useful in the amendment of contaminated soil. These plant waste contain complex molecules e.g. cellulose, hemicellulose and lignin, and anti-nutritional compounds such as cyanogenic glycosides, oxalates, phytates and trypsin [24]. Hence, the peels/wastes of some plants, which contain adequate nutrients to replenish that which is lacking in contaminated soil environment, can be utilized to stimulant the growth of inherent microbes for the degradation of the contaminant in the soil via biostimulation process [12].

Citrullus colocynthis, commonly known as bitter apple or bitter melon, is a fruit crop that belongs to the family *Cucurbitaceae* [25] *and al*so known as *Colocynthis vulgaris* (schrad) or *Colocynthis officinalis* (Schrad) [24]. *Citrullus colocynthis* peels or husks, generated manually or mechanically when processing *Citrullus colocynthis* seed for cooking purposes [26], are disposed or burnt indiscriminately to cause soil and air pollutions, respectively [27]. Nigeria been the largest producer of *Citrullus colocynthis* in the African [28] has most of her land polluted with *Citrullus colocynthis* peels, especially in the Northern Nigeria where *Citrullus colocynthis is mostly grown.* The peels of *Citrullus colocynthis* peels contain proteins (15.5%), carbohydrates (69.59%), lipid (2.05%), fibre (6.07%) and ash (6.79%) [29]; also its minerals content (percentage) 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) [24]. However, according to Ajayi and Lateef, [24], the major anti-nutrients of *Citrullus colocynthis* 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) [30]. This indicates that *Citrullus colocynthis peels have adequate nitrogen (N), phosphorus (P), carbohydrate and other minerals that microorganisms can utilize to*

survive in soil environment such as spent oil-contaminated soil, which is deficient in these important nutrients. Therefore, this study investigates the use of *Citrullus colocynthis* peels, from the Northern Nigeria, and other plant waste for the effective degradation of spent oil in soil as compared to natural attenuated method.

2. MATERIALS AND METHODS

2.1 Soil and Plant Waste Sampling

Uncontaminated soil was collected from a farmland free of oil contamination at Sheda Science and Technology Complex (SHESTCO) community in 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; *Citrullus colocynthis* peels were obtained at melon processing mill, Dabi; Sawdust was collected from the timber market Gwagwalada; fertilizer was purchased at Gwagwalada market.

2.2 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 ºC in the oven [31] 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 to reduce the cyanide content that would endanger the inherent microorganisms [32].

2.3 Microcosm Setup

About 2 kg of the soil samples were weighed into 20 plastic bucket, consecutively 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 weekly for adequate moisture content and mixed adequately to increase aeration of the soils. The plastic buckets were labelled A to J and treated with the biostimulants as indicated. Bucket A was treated with 400 g of NPK (15:15:15) fertilizer; B was treated with 400 g of cassava peels and C was treated with 400 g *Citrullus colocynthis* peels. Bucket D contained 400 g Sawdust; E contained 200 g of *Citrullus colocynthis* peels and 200 g of cassava peelings. In Bucket F, 200 g of cassava peelings and 200 g of Sawdust were added while G was treated with 200 g of *Citrullus colocynthis* peels and 200 g of Sawdust. Bucket H contained 133.33 g of cassava peeling, 133.33 g of *Citrullus colocynthis peels* and 133.33 g of Sawdust; Bucket I contained only oil, no treatments (control) whereas, J contained auto-claved soil samples poisoned with 400 ml of sodium azide solution at 6.5%, as it inhibits the growth of microorganisms [33]. The treatments were duplicated to give 20 microcosms.

2.4 Sampling

Sodium azide was added in Microcosm J, after 20, 40, 60, 80, 100 and 120-day 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 biodegradation by the inherent microorganism following biostimulation with the different plant wastes. Approximately 4 g of soil were sampled from different portions of the plastic bucket to form a composite sample. This was done for all the 20 plastic buckets.

2.5 Extraction

The samples were analysed for the extent of biodegradation of spent oil via cold extraction method. The residual oil in each sampled soil was cold extracted following the method of Nna Orji [9] using dichloromethane as solvent. About 20 weighing boats were labelled, weighed and approximately 2 g of the soil from each of the 20 microcosms and 2 g of Na_2SO_4 were weighed in to each boat. These were properly mixed and transferred quantitatively into a burette blocked previously with glass wool. The burette was also blocked with extra glass wool after the transfer of the soil mixture. Then, extraction was carried out with 3 ml of dichloromethane. The extraction continued until the glass wool at the tip of the burette was free of oil. The weighing boats used for the extraction were weighed again following complete evaporation of the solvent. Then, the dry weight of the oil was determined and the residual oil in 1 g of the soil was determined.

Gain in weight of flask (mg) = (weight Bijou bottle and residue after evaporation of extraction solvents) – (weight of empty Bijou bottle)

$$\text{Residual oil (g/g)} = \frac{\text{gain in weight of flask (g)}}{\text{weight of wet solid (g)}}$$

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.

3. METHODS 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.

4. RESULTS AND DISCUSSION

The result of the mean residual oil extracted from the twenty (20) microcosms setup for the biodegradation of spent oil in soil following amendment and incubation for 180 days is shown in Fig. 1.

The presence of spent oil in the soil resulted to the unavailability of soil nutrient to and poisoning of the soil inherent microorganisms, which incapacitated the microbes and hindered them from degrading the oil contaminant. Biostimulation enhances the growth and survival of the inherent microorganisms so they can use the spent oil as their sole source of food, carbon and energy. During the incubation period of 180

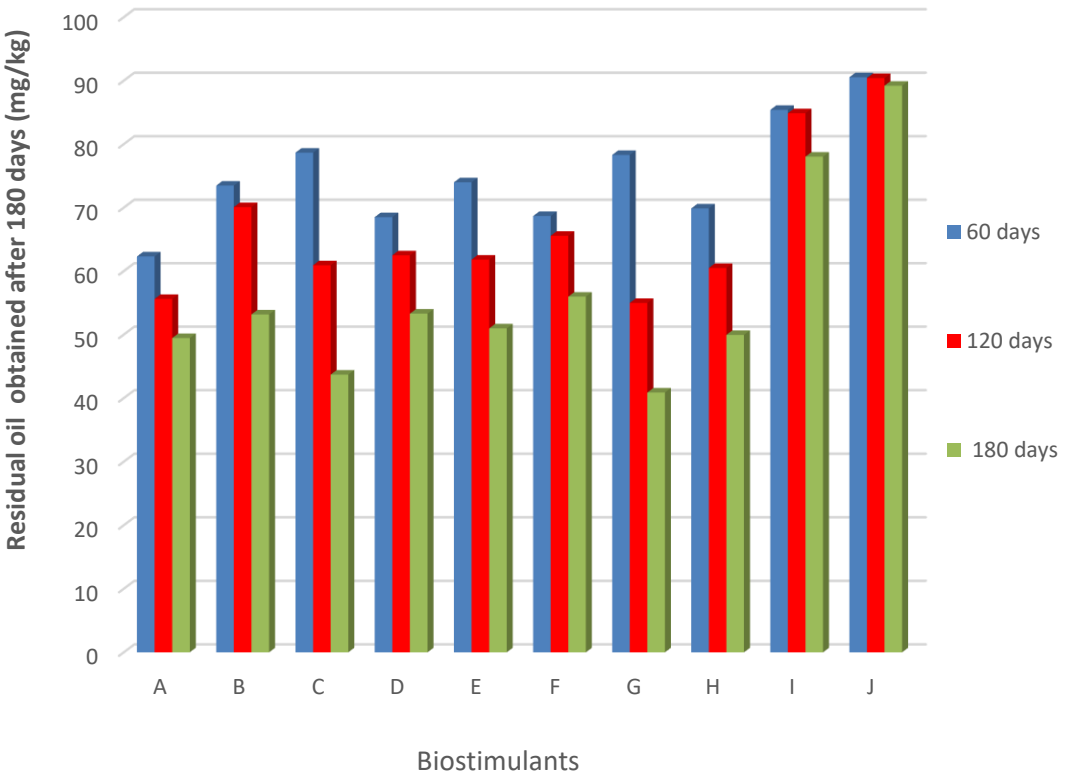

Fig. 1. Residual oil (mg/ kg) extracted following biostimulation with plant waste for 180 days

days, the mean residual oil extracted from the microcosms biostimulated with individual plant waste ranged from 43.7±5.27 to 78.66±1.87 mg/kg. The mean residual oil extracted from microcosms biostimulated with paired plant wastes, ranged from 40.88±4.21 to 78.3±0.09 mg/kg within the 180 days of study while the mean residual oil extracted from the microcosms stimulated with a mixture of three plant wastes, ranged from 49.95±8.20 to

69.88±6.53 mg/kg. However, the natural attenuation microcosms, which had no plant waste as biostimulant to improve the nutrients of the soil, had the highest mean residual oil extracted from the microcosm and ranged from 78.03±1.45 to 90.54±0.14 mg/kg. This indicates that the inherent microorganisms were not able to degrade much of the spent oil contaminant and may be due to the poisoning effect of spent oil on the soil organisms [34], which led to the death of the soil organisms [35] and hence little or no biodegradation took place.

From the ANOVA analysis, the interaction between the days of biostimulation and the stimulants accounted for 10.3 % of the total variation in the result obtained after extraction following biostimulation with a *P* =.005 showing a significant difference. Also, about 28 % variation in the quantities of the residual oil obtained was caused by the days allowed for the biostimulation of the inherent microbes while the stimulants used for the biostimulation accounted for 55.2 % of the total variation in the result, with *P*=.000. These effects were extremely significant, implying that for effective biodegradation of oil contamination in the soil, biostimulant 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 [36] into non-toxic compounds.

4.1 Biodegradation of Spent-oil After 60-day Biostimulation

The effectiveness of *Citrullus colocynthis* peels, other plant wastes and natural attenuation for the biodegradation of the spent oil in soil during an incubation period of 60 days is presented in Figure 2. During the first 60-day incubation period, there was appreciable reduction of the amount of the residual extracted from the biostimulated microcosms, showing the commencement of biodegradation. The mean residual oil extracted from biostimulated microcosms after 60 days ranged from 62.31±2.50 mg/kg of with NPK fertilizer) to 78.66±1.87 mg/kg *Citrullus colocynthis* peels s while the residual oil from the control microcosms ranged from 85.4±6.05 to 90.54±0.14 mg/kg.

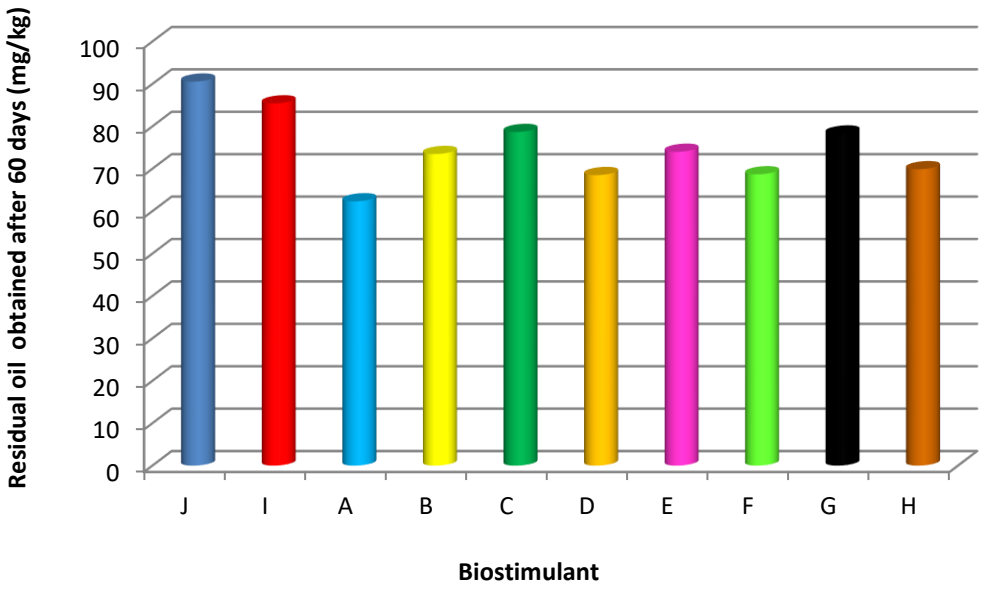

Fig. 2. Residual oil (mg/ kg) extracted during biodegradation of spent oil following biostimulation for 60 days

Multiple comparisons test was carried out to ascertain the significance difference between the performance of the control with & without NaN_3 and each of the microcosms biostimulated with plant wastes for the biodegradation of the spent oil in soil at α = 0.05. The test revealed that after 60 days of incubation, the residual oil of 62.31±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 peels + *Citrullus colocynthis* peels + Sawdust microcosms, were significantly lower than 90.54±0.1414 mg/kg extracted from natural attenuated microcosms with NaN_3. As indicated, *p*-values<0.001, *p*=.01, *p*=.01, *p*=.02 and *p*=.01 respectively at 0.05 level of significance were obtained. These performances of the biostimulated microcosms indicated the enhancement of the growth 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. This showed that microorganisms are important for the degradation of spent oil in the soil. In addition, the quantity of the reduced residual spent oil in the biostimulated microcosms showed that biodegradation occurred via the inherent microorganisms that were stimulated due to the available nutrients in the stimulants.

The performance of the biostimulants in their respective microcosms was compared with the natural attenuated microcosms without NaN_3 after 60-day of incubation. The test revealed that the residual oil of 68.50±7.35 mg/kg from the microcosms biostimulated with the inorganic NPK fertilizer significantly enhanced the biodegradation of the spent oil in the soil better than the natural attenuation without NaN_3. Hence, the biostimulation of the inherent microbes with NPK fertilizer was the only stimulant that enhanced the biodegradation of the spent oil better than the control without NaN_3. This implies that after the 60 days of biostimulation, *Citrullus colocynthis 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 and thereafter, release their inherent nitrogen and phosphorus [37] into the soil environment for adequate biostimulation of the inherent microorganisms and enhanced biodegradation of the spent oil in the soil. In addition, NPK was able to release it nutrient into the environment within 60 days for the growth and survival of the inherent microbe.

4.2 Biodegradation of Spent-oil After 120-day Biostimulation

The quantities of residual oil extracted from microcosms treated with plant wastes after 120 days is shown in Fig. 3.

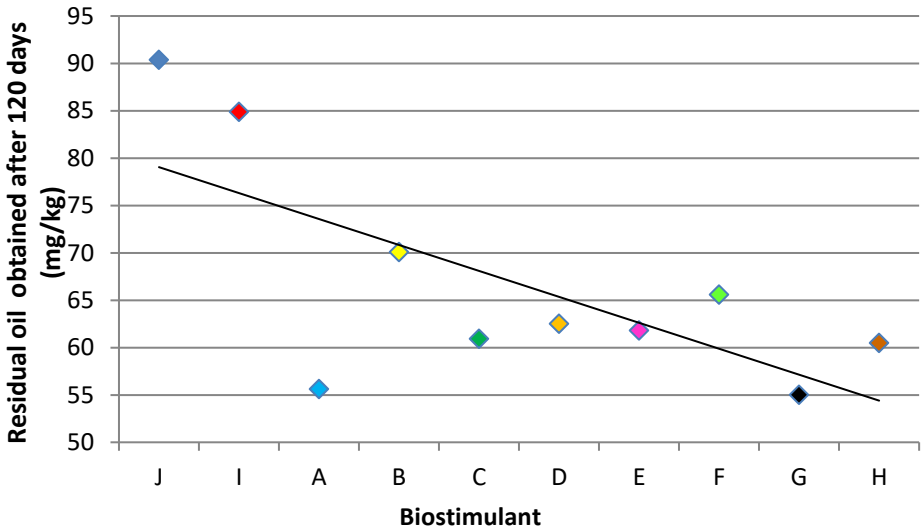

Fig. 3. Residual oil (mg/ kg) extracted during degradation of spent oil in soil following treatment with plant waste after 120 days

The mean residual oil extracted after 120 days from the biostimulated microcosms ranged from 54.99±6.91 mg/kg of *Citrullus colocynthis* peels + Sawdust to 70.08±1.27 mg/kg of cassava peelings, whereas, the mean residual oil of the control microcosms, ranged from 84.87±0.90 to 90.4±0.10 mg/kg. The order of the quantities of the mean residual spent oil extracted from the amended microcosms was *Citrullus colocynthis peels* + Sawdust< NPK Fertilizer< Cassava

peelings + *Citrullus colocynthis peels* + Sawdust < *Citrullus colocynthis peels* < *Citrullus colocynthis peels* + Cassava peeling< Sawdust< Cassava peelings + Sawdust< Cassava peeling. However, the mean residual spent oil of 90.4±0.0990 mg/kg extracted from the control microcosms with NaN_3 was significantly the highest mean after 120 days of incubation. Hence, the amendments significantly enhanced the biodegradation of the spent oil contaminant in the contaminated soil better than the natural attenuation with NaN_3. The results showed the significance of the presence, viability and ability of inherent microorganisms to use up the nitrogen and phosphorus released by the biostimulants for their biostimulation and eventually the degradation and reduction of the quantity of the spent oil extracted after 120 days of incubation. Also, the residual oil extracted from the control without NaN_3, after 120 days of incubation, was significantly higher than those extracted from the microcosms biostimulated with NPK, *Citrullus colocynthis*, Sawdust, *Citrullus colocynthis* + Cassava peelings, *Citrullus colocynthis* + Sawdust and Cassava peelings + *Citrullus colocynthis* + Sawdust with *p*-values< 0.001, *p*=.004, *p*=.009, *p*=.006, *p*=.034, *p* <0.001 and *p*=0.003, respectively at 0.05 level of significance. This shows that all the biostimulants except cassava peelings were able to biostimulate the inherent microbe after an incubation period of 120 days. Thus, they have enhanced the biodegradation 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 biostimulation of the inherent microbes for the degradation of the spent oil [32].

4.3 Biodegradation of Spent-oil After 180-day Biostimulation

Figure 4 shows the performances of *Citrullus colocynthis peels* and other plant wastes used for the stimulation of the inherent microorganisms for the biodegradation of the spent oil in soil during an incubation period of 180 days.

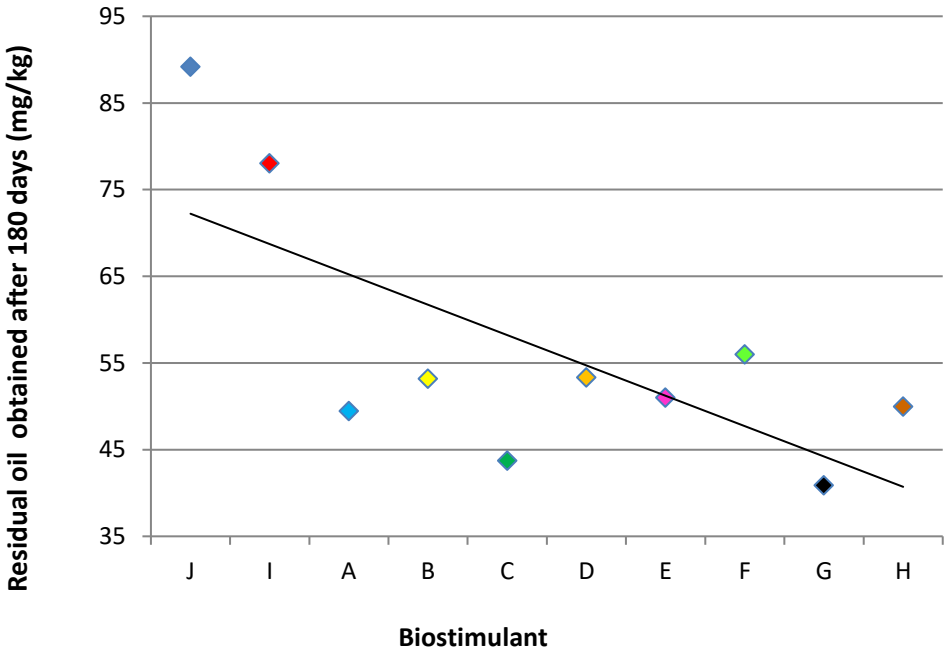

Fig. 4. Residual oil (mg/ kg) extracted from waste oil artificially contaminated soil following treatment with plant waste after 180 days

The order of the amount of the extracted residual oil from each of the microcosms after 180-day incubation period was G< C< A< H< E< B< D< F< J< I. At the end of 180 days of incubation, the lowest mean extracted residual oil was 40.88±4.21 mg/kg, extracted from *Citrullus colocynthis peels* + Sawdust, followed by a mean of 43.70±5.27 mg/kg extracted from the microcosms stimulated with only *Citrullus colocynthis peels* and thirdly by 49.45±5.73

mg/kg from NPK biostimulated microcosms. After 180 days, the range of the extracted residual oil from the biostimulated microcosms was 40.88±4.21 mg/kg of the *Citrullus colocynthis peels* + sawdust to 55.98±3.50 mg/kg from the microcosms biostimulated with cassava peelings + Sawdust. Meanwhile, the extracts from the control microcosms ranged from 78.03±1.45 to 89.2±0.07 mg/kg.

The quantities of spent oil extracted from the microcosms depicted the performances of the varying stimulants used to biostimulate the inherent microbe for the biodegradation of the spent oil in soil. From the results, there was no significant difference between the performances of one plant waste with the others, implying that they similarly enhanced the biodegradation of the spent motor oil by the inherent microorganisms. Hence, the microcosm containing only *Citrullus colocynthis peels* and those containing fractions of *Citrullus colocynthis peels* performed just like the other plant wastes use for the biodegradation of the spent oil in the different microcosms.

In addition, there was extremely significant difference between the performance of the biostimulated microcosms and the natural attenuation containing NaN_3, with *p*-values <0.001 and at 0.05 significant level. The indication is that, the plant waste in the biostimulated microcosms significantly enhanced the performance of the microbes present in the soil for the biodegradation of the spent oil in the soil and the absence of microorganism as shown in the natural attenuation microcosms with NaN_3, showed no degradation of the spent oil. Hence, the nitrogen components of the biostimulants released into the soil increased the activities of the microbes for the degradation and reduction of the spent oil in the soil better than in the controls without microorganisms and any biostimulant [38]. The presence and effect of the sodium azide in the contaminated soil, which resulted in the death of the microbes, reduced the soil enzyme activity and microbial population [39,40], and did not really differ or made any difference compared to when the sodium azide was not added. This proves that the inherent microbes were not actually active but dormant and incapacitated due to the presence and poisoning effects of the spent motor oil in the soil. The contamination by spent oil caused harm, instability, discomfort to the soil organisms [41], and changes the physical, chemical and biological systems of the soil flora [42]. More so, the increased aeration and moisture content to ensure maximal survival of the soil microbes, had no significant effect in improving and ensuring adequate growth of the inherent microorganisms in the natural attenuated microcosms without NaN_3.

4.4 Monthly Performance of Each Biostimulant

Multiple comparisons test was carried out to compare the extracted residual oil from one month with those from the rest of the months following biodegradation of the spent oil and enhancement by each stimulant.

4.4.1 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. The conventional NPK fertilizers release their nutrients faster and have high dissolution rates in the soil than the NPK slow releasing fertilizer that are coated [43,44]. Thus, the convention NPK fertilizer used in this study dissolved 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 results. The quantity of spent oil extracted following biostimulation with convention NPK after 180 days was significantly lower than that extracted after of 60-day incubation period. Therefore, the ability of NPK fertilizer to enhance the nutritional value of the soil for adequate biodegradation after 180 days of biostimulation was significantly higher than its performance after 60 days. With p=.0256, it indicates that time played a major role in the biostimulation by NPK, as an inorganic fertilizer

4.4.2 Microcosm B

The mean residual oil extracted from the microcosms biostimulated with cassava peel ranged from 53.18±3.57 to 73.47±7.02 mg/kg extracted after 180 and 60-day incubation period, respectively. The enhancement of the inherent microbes by cassava peels for the biodegradation of the spent oil in the soil after 180 days was significant better than those after 60 and 120 days with $p<0$.001 and p=.0035 respectively. This could be attributed to the low level of nitrogen and high cyanide content of the peels, which could be damaging to the inherent

microbe [32,44]. The result also showed 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 biostimulation of the inherent microbe..

4.4.3 Microcosm C

The results from Fig. 2- 4 shows a progressive reduction in the quantity of residual spent oil extracted from the microcosms biostimulated with *Citrullus colocynthis* following incubation for 60 – 180 days. 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 extracted from the microcosm after 180 days was significantly lower than that after 120 days, which was significantly lower than that after 60 days of biostimulation. This means that the enhancement of the microbes for the biodegradation of the spent oil in the soil by *Citrullus colocynthis peels,* was significantly better after 180 days than after 60 and 120 days of biostimulation at $p<0.05$ and its performance after 120 days was significantly better than after 60 days. The high nitrogen content of *Citrullus colocynthis peels* was released after the decomposition of *Citrullus colocynthis peels*, enabled the growth of the inherent microbes and increase the microbial activities for the biodegradation of the spent oil into non-toxic compounds [38]. Therefore, the biodegradation of the oil contaminant with *Citrullus colocynthis peels* started from the beginning of the biostimulation and made a significant improvement in the activities of the microbes towards reducing and degrading the spent oil contaminant.

4.4.4 Microcosm D

From the results, shown in Fig 2-4. At the end of the incubation period, the mean residual spent oil extracted after 180 days of biostimulation was significantly lower than that extracted after 60 days. However, there was no significant difference between the quantities of residual oil extracted after 60 and that after 120 days and between 120 and that after 180 days of biodegradation. Hence, biostimulation 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 $p=.008$ at 0.05 significant levels. The performance of the biostimulant may be due to the long-time required for the complex molecule of the sawdust to decay and let down its nutrient for uptake by and growth of the inherent microbe.

4.4.5 Microcosm E

Citrullus colocynthis peels and Cassava peels worked in synergy in this microcosms for the degradation and reduction of spent oil in the soil. The results in Fig. 2-4 showed that there was a gradual reduction in the quantity of residual oil extracted from the microcosm during the 180-day incubation period. This may be due to the released nutrient to the inherent microbes for the biodegradation of the spent oil from *Citrullus colocynthis peels* + Cassava peelings. The synergy in performance for the reduction of the extracted residual oil between the paired plant wastes after 120 and 180-day incubation period, were significantly lower than that 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 *Citrullus colocynthis peels* [30] and cassava peelings [32,43] before making any contributory biodegradation of the spent oil, thereby reducing the residual oil in the soil. It may also be due to the lower nitrogen content of cassava peels, 1.69 % [45] as compared to that of *Citrullus colocynthis peels* 15.5 % [29].

4.4.6 Microcosm F

The results of the synergy between cassava and sawdust in the biodegradation of spent oil in soil is shown in Fig. 2-4, revealed that the residual oil of 68.68±2.55 mg/kg, extracted after 60 days of biodegradation 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 biodegradation. Hence, the reduction of the quantity of oil extracted from the microcosm after 180 days was lower than that after 60 days with $p=.0275$. This performance in synergy lower than their performance when used individually. It could be there was antagonistic effect by the stimulants on the inherent microbes. More so, the acidic nature, cyanide and low nitrogen content of the cassava peelings may have caused a reduction in growth of the microbes [45], hence effected the quantity of spent oil extracted during the process. The time need to decompose the matrix of the sawdust may have as well affected the

rate at which nutrient are released from the sawdust for the enhancement of the growth of the microbes.

4.4.7 Microcosm G

The combined interaction between *Citrullus colocynthis peels* + sawdust for the enhancement of the growth of the inherent microbe to biodegrade the spent oil and reduce the quantity of the residual oil extracted, yielded a lowest mean residual oil, 40.88±4.21 mg/kg, at the end of the 180-day biodegradation process. This synergy produced a progressive significant difference in the reduction of the residual oil extracted from the biostimulated microcosms and could be due to the high nitrogen content of the *Citrullus colocynthis peels*, which must have increased the activities of the inherent microorganisms in the soil for biodegradation and eventual reduction of the quantity of the residual oil extracted. The effectiveness of the synergy between *Citrullus colocynthis peels* + sawdust in the reduction of the residual oil was better than their individual performance in their respective microcosms.

4.4.8 Microcosm H

This microcosm contains *three plant waste namely Citrullus colocynthis* peels*,* sawdust and cassava peels*. Citrullus colocynthis* has high content of nitrogen, *Citrullus colocynthis* peels and sawdust have complex cellulose structure whereas *Citrullus colocynthis* peels and cassava peels contain cyanide. From the results, the residual oil, 49.95±8.20 mg/kg, extracted after 180 days of biostimulation with the mixture of these three plant waste, differed significantly from that extracted after 60 days of incubation, *P*-value<0.001 at 0.05 significant level. This significant performance of the three-plant waste in the reduction of the quantity of residual oil extracted between 60 – 180 days may be due to time required to decompose the complex molecule of *Citrullus colocynthis* and the sawdust, handle the residual cyanide in the *Citrullus colocynthis peels* and cassava peels and thereafter the microbes absorb the let-down nutrients.

At the end of the 180 days of biodegradation following biostimulation with all the microcosms containing combined biostimulants, *Citrullus colocynthis* peels + sawdust had the lowest mean extracted residual oil of 40.88±4.21 mg/kg followed by *Citrullus colocynthis* peels + Cassava peelings + sawdust with 49.95±8.20 mg/kg and thirdly by *Citrullus colocynthis* peels + Cassava peelings with 50.98±1.59 mg/kg. The microcosms with the highest extracted mean residual oil among the combined biostimulant was *Citrullus colocynthis* peels + sawdust with 55.98±3.50 mg/kg. However, from the multiple comparison test, there was no statistical 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 biodegradation process, $p>0.1$ at $\alpha= 0.05$. This indicates an overall similarity in their performances and effectiveness in their ability to reduce the amount of residual oil extracted every 60 days of incubation period.

5. GC/MS ANALYSIS OF RESIDUAL OIL

The GC/MS analysis of the spent oil from car obtained from the mechanic workshop identified 21 compounds [9]. 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 [9]. 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 [9].

Fig. 5. Shows the chromatogram of the extract from microcosms G, which contains contaminated soil biostimulated with *Citrullus colocynthis peels* and Sawdust.

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 biostimulation with *Citrullus colocynthis peels* and Sawdust in the soils artificially contaminated with spent oil.

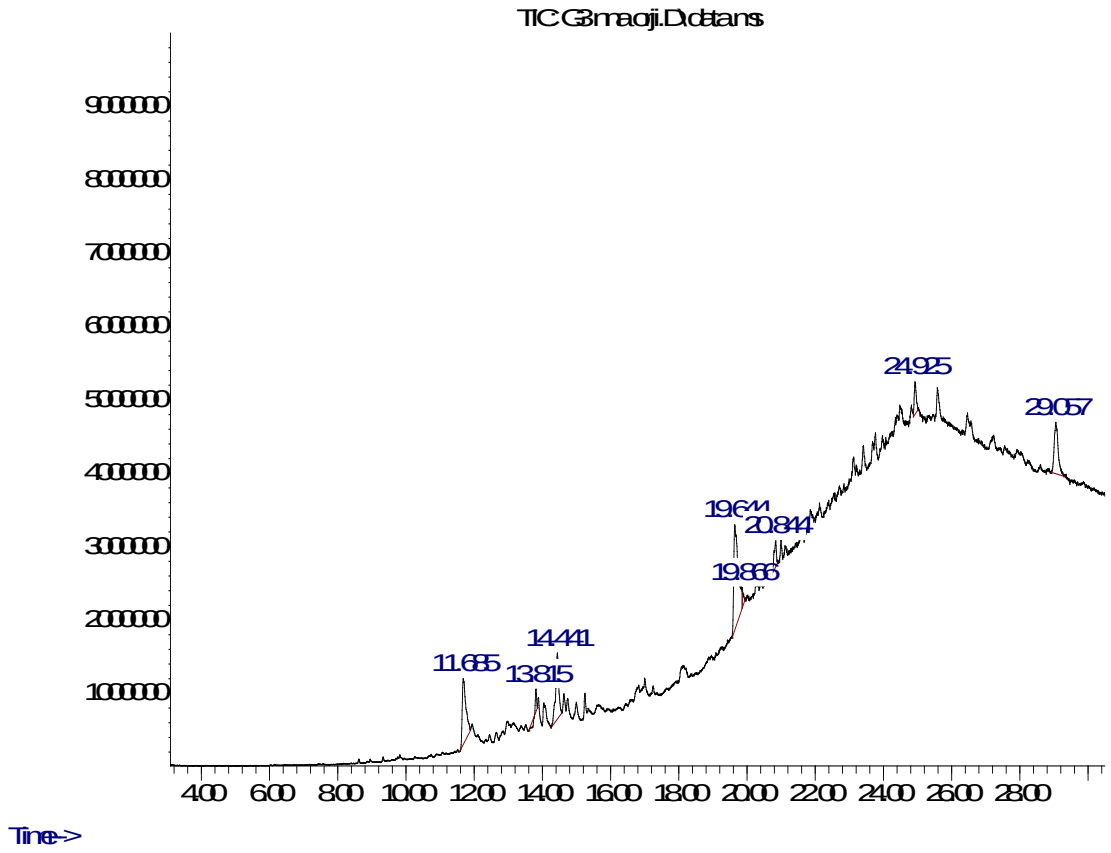

Fig. 5. Chromatogram of residual oil extracted after 180-day biostimulation with yellow *Citrullus colocynthis peels* + Sawdust peelings for 180 days

Table 1. Compounds identified in residual oil following biostimulation with *Citrullus colocynthis 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-; three nitrogen-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 five Oxygen-containing compounds were identified in the extracted residual oil as shown in Fig 6.

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-(5Methoxy) phenylpropionic acid while 2 mono-aromatic compound identified were Diethyl Phthalate and Picolinyl 6,9-octadecadienoate

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 [46], serum [47], breast milk [48], etc. These metabolites have been linked to type II diabetes, overweight/obesity, and asthma, poor semen quality in men and increased pregnancy loss in women [49].

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-dihydro-phenanthrene had the least. Diethyl Phthalate and 2-Amino-5-isopropyl-8-methyl-1-azulene-carbonitrile had the second and third highest peak area percentage, respectively. The chromatogram of the residual oil extracted from microcosm C following biostimulation with *Citrullus colocynthis peels* after 180 days is shown in Fig. 8.

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

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 Tetracosyl trifluoroacetate, 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 Tetracosyl trifluoroacetate (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,

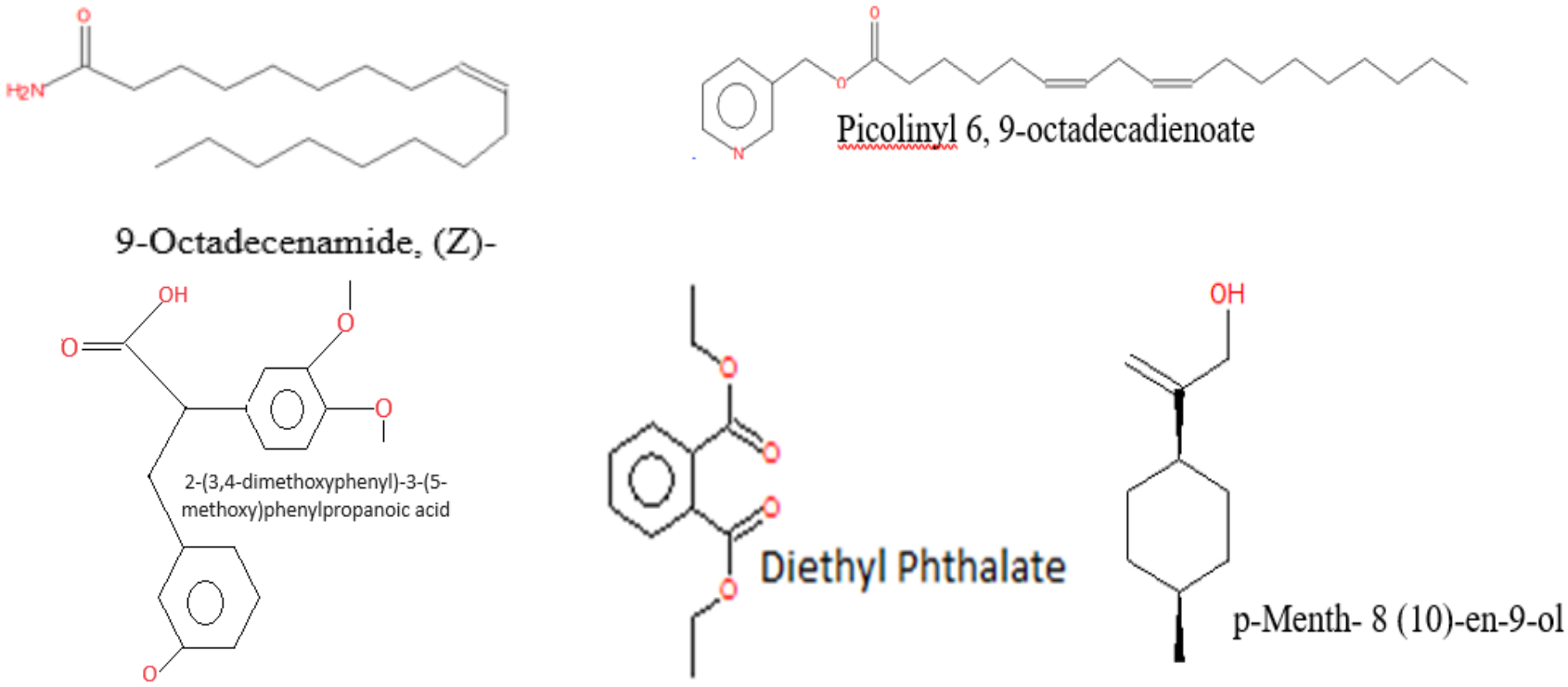

Fig. 6. Oxygenated compounds identified in the residual extracts from microcosm G

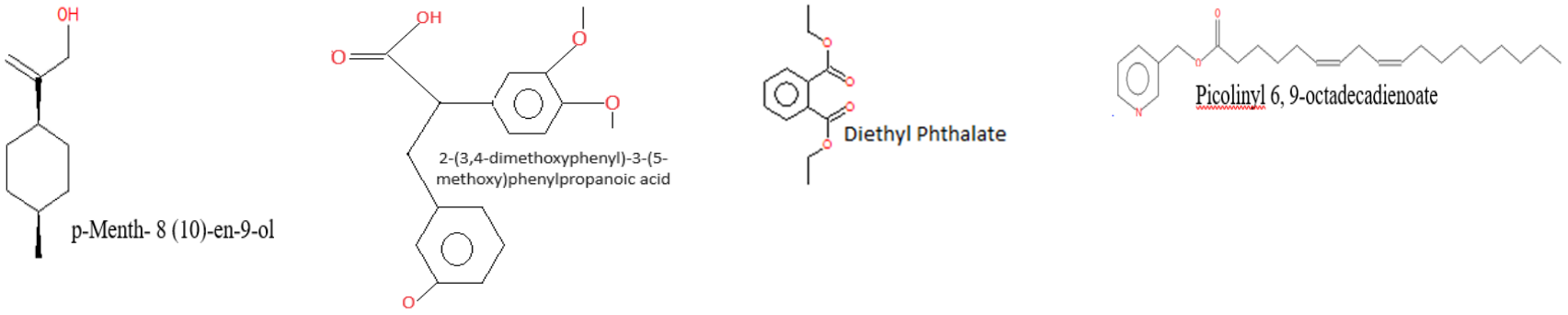

Fig. 7. Oxygenated compounds identified in the residual extracts from Microcosm G

Table 2. Compounds identified in residual oil following biostimulation with *Citrullus colocynthis peels* for 180 days

S/NO	Compound Name	Molecular formula	Molecular weight (g/mol)	Area %
1.	2-Amino-5-isopropyl-8-methyl-1azulenecarbonitrile	$C_{15}H_{16}N_2$	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.	Tetracosyl trifluoroacetate	$C_{26}H_{49}F_3O_2$	450.00	1.47

Pentadecane, 2,6,10,14-tetramethyl and 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, two Nitrogen containing compounds were identified*:* 2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile and 4-[3-Ethoxypropylamino]benzo-1,2,3-triazine. More so, two halogenated compounds named Tetracosyl trifluoroacetate and 1-Bromo-11-iodoundecane were identified.

The aromatic compounds found after biostimulation with *Citrullus colocynthis peels* are shown in Fig 9.

The only mono-aromatic and tri-aromatic compounds identified were 1,5,6,7-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 biostimulated with *Citrullus colocynthis peels*, C, and *Citrullus colocynthis peels* & sawdust, G, about 20 % and 50 % of the compounds were aromatic compounds, respectively. However, according to Nna Orji [9] 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 biodegradation. 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 [9]. In aquatic environment, health hazards may result due to bioaccumulation in aquatic animal, and may lead to health risk in the body of man when these aquatic animals are consumed.

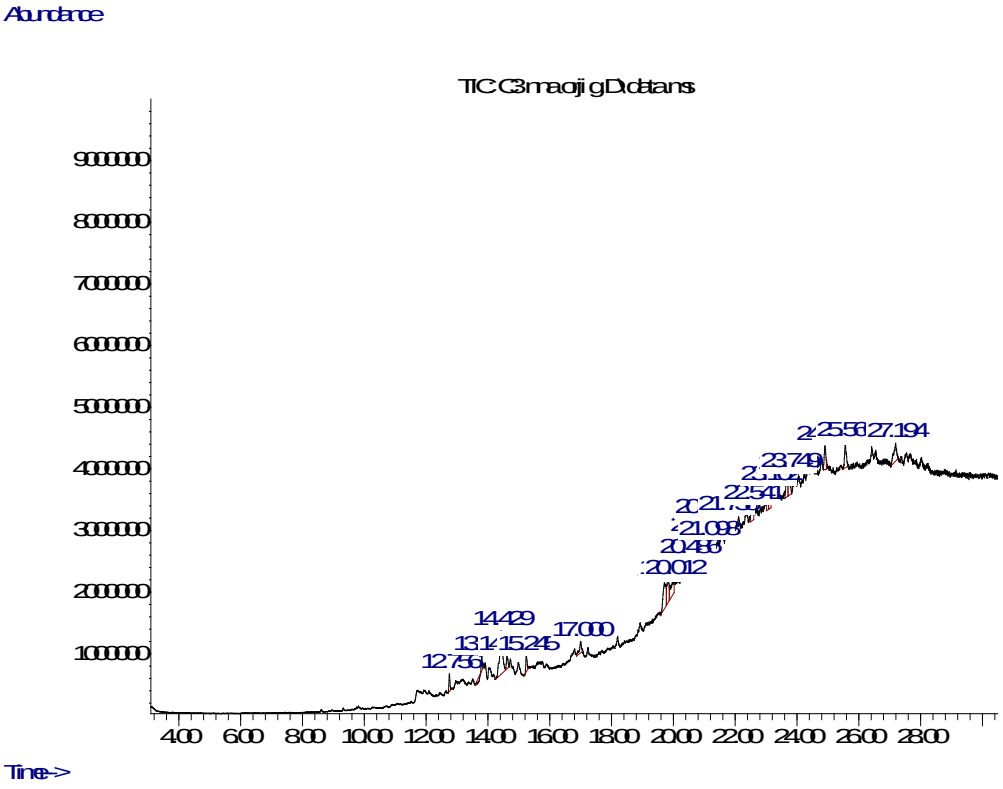

Fig. 8. Chromatogram of residual oil extracted after 180-day biostimulation with *Citrullus colocynthis* peels

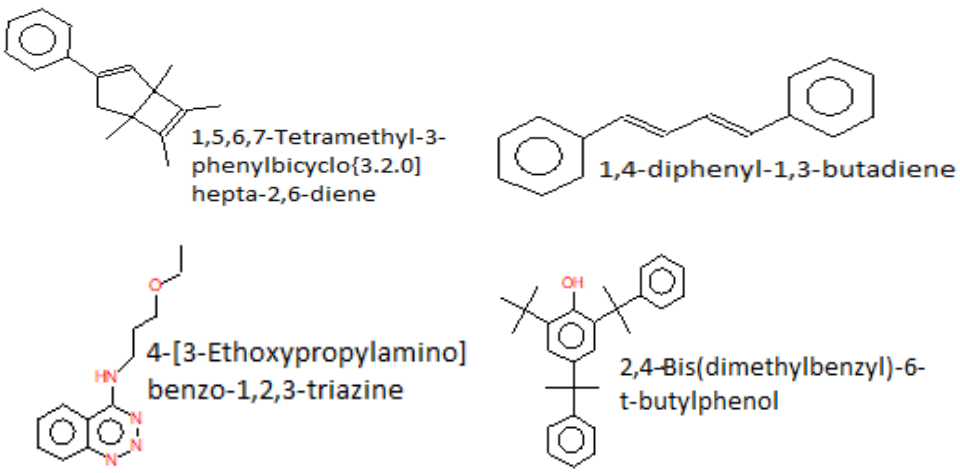

Fig. 9. Aromatic compounds identified in the residual extracts from Microcosm C

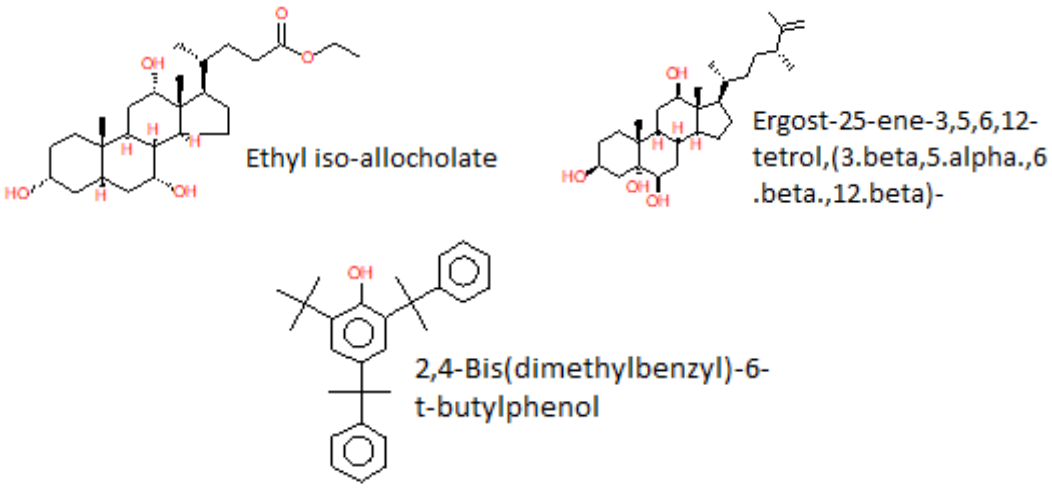

Fig. 10. Chemical structure of compounds identified to be from *Citrullus colocynthis peels*

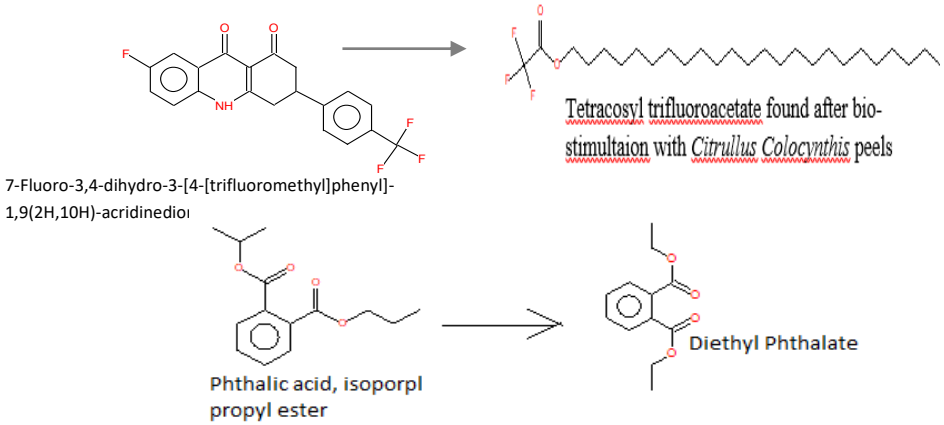

Fig. 11. Chemical transformation

Some compounds identified after biostimulation with *Citrullus colocynthis peels* were different from those compounds identified in spent oil, indicating that they were inherent in *Citrullus colocynthis peels*. The chemical structure of these compounds are shown in figure 10 and are: Ethyl iso-allocholate, Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5.alpha.,6.beta.,12.beta.)- and 2,4-Bis(dimethylbenzyl)-6-t-butylphenol

In addition, some compounds identified before may have been transformed after biodegradation process are shown in Fig 11. Phthalic acid, isoporpl propyl ester and 7-Fluoro-3,4-dihydro-3-[4-[trifluoromethyl]phenyl]-1,9(2H,10H)-acridinedione were identified in spent oil before biostimulation while Bis(2-ethylhexyl)phthalate and Tetracosyl trifluoroacetate were identified after biostimulation with Citrullus *colocynthis peels* + Sawdust and Citrullus *colocynthis peels*, respectively.

A Volatile Organic Compound (VOC), 2-Amino-5-isopropyl-8-methyl-1 azulenecarbonitrile, was found in spent oil before biostimulation [9], with area percent of 16.70; after bio- stimulation with *Citrullus colocynthis peels* + Sawdust, 16.35% was observed; with only *Citrullus colocynthis peels*, it had 15.42 % after 180 days of stimulation. It causes cancer in the colon, breast, prostate, etc. [50]. The 9-octanamide identified 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 [48].

6. CONCLUSION

In conclusion, the biodegradation of spent oil in soils was effective and efficient after biostimulation with *Citrullus colocynthis peels*

and other plant waste for 180-day incubation period. The biodegradation depended on the number of days allowed for biodegradation to occur, inherent nutrient and the abilities of plant waste to release their nutrient. These enhanced the growth of the inherent microorganisms, which used the oil contaminant as their major source of food and carbon and led to the reduction of the extracted residual oil following periodic biodegradation. In the study, all the microcosms, which contained the leguminous wastes, *Citrullus colocynthis peels*, reduced the quantities of the oil extracted from the biostimulated soil microcosms progressively. The biostimulated microcosms had their spent oil degraded and reduced more than natural attenuated microcosms and lower molecular weight compounds formed. Therefore, *Citrullus colocynthis peels* and other plant waste investigated can be used to biodegrade the spent oil in soils to reclaim lands for farming and other purposes. Hence, pollutants from the Northern Nigeria can be used to treat spent or crude oil pollutant in the Southern Nigeria.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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