CRUDE OIL POLLUTED SOIL AMENDMENT USING DRIED FRUIT WASTE IMPROVES SOIL PHYSICOCHEMICAL INDEX AND ENZYME ACTIVITIES; A LABORATORY CONTROLLED STUDY WITH POTENTIAL FOR FIELD TRIAL

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ABSTRACT

Purpose: The search for a cheap source of crude oil bioremediation agent is in continuum. Waste fruits are a national issue due to increasing volumes of poor storage facilities, urbanization, population growth, and poor standards of living; however, they could besides be employed for several beneficial purposes. The aim of this study was to examine the effect of dried waste fruits on physicochemical index and enzyme activities of crude oil contaminated soil.

Method: Crude oil defiled soil was obtained from Oleh/Olomoro flow station in Delta State and grouped into 5 followed by amendment with various combinations of the dried waste fruits (Orange, Pineapple and Water melon), obtained from Merogun wasteland, Warri Delta State. Group 1 is the unamended polluted soil. Groups 2 and 3 comprised of a polluted soil amended with 20% and 40% w/w respectively for one week; Groups 4 and 5 comprised of a polluted soil amended with 20% and 40% w/w respectively for three week. Group 6 is a non-polluted soil collected from a virgin land along Abraka-Aragba express way.

Result: The results after three weeks soil amendment (169.34±1.15 mg/l) showed that there was a significant reduction in the total hydrocarbon content compared to the unamended soil (242.98±3.70 mg/l). Soil physiochemical properties also improved significantly ($P<0.05$). The result further revealed a marked increase in soil microbial populace in amended soils relative to the unamended group.

Conclusion: The results suggest that nutrient supplementation using the dried waste fruits was effective in the improvement of crude oil contaminated soils.

KEYWORDS:-Crude oil pollution, Bioremediation, Dried waste fruit amendment, Orange Pineapple Watermelon waste fruits, Soil physicochemical index, Enzyme activities.

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Introduction

Over the past 50 years, crude oil exploration and production have significantly boosted Nigeria's economy. However, the Niger Delta States of Nigeria are currently grappling with a number of serious environmental problems, including accidental oil spills that cause environmental issues within oil-producing communities. Operational practices, equipment failure, oil pipeline vandalism/bunkering, tanker accidents, and calamities can all be blamed for oil spills in this area. Due to this unfortunate event, refined petroleum products and crude oil have been released into the atmosphere and waterways [1]. Studies on the effects of crude oil on soil have shown that one effect is a decrease in pH, which leads to a reduction in soil fertility [2].

The ability of naturally existing organisms to decontaminate hydrocarbon-polluted environments by converting hazardous chemicals into harmless or volatile compounds is receiving positive interest from researchers [3-5]. Oil spill-induced inequality or unevenness is the main factor holding limiting the bioremediation of petroleum pollution in ambient or native environment, which is a process that appears to take a long time. Numerous factors, such as a deficiency of necessary nutrients like nitrogen, limit the rates at which petroleum hydrocarbons degrade. Thus, the addition of inorganic or organic nutrients high in nitrogen (bio-stimulation) is an efficient method for accelerating the bioremediation process.

Fruit waste is an old and modern problem in Nigeria, linked to poor storage facilities, high fruit perishability, poverty, agricultural land insecurity and low living standards and this have led to indiscriminate dispersing of several of them by fruit sellers and farmers [6]. The search for cheaper and greener options to ameliorate crude oil degradation is in continuum. Organic wastes in the form of animal and plant materials such as rice husks, coconut husks, Moringa oleifera and soybeans, cow, pig and goat manure are some of the options that have been explored at recycling, some fruit wastes such as banana peels, oranges and watermelons have also been reported for trials with regards to bioremediation of crude oil pollution. At the time of our study, the studies conducted mainly focused on the use of single fruit waste, there is limited literature examining the use of several dried fruit mix combinations made from fruit wastes. This study therefore aimed at the exploration of the potential of dried fruit waste mix made from a combination of putrefied fruits and fruit peels (oranges, watermelons and pineapples), readily available from wastelands and fruit sellers and are known to constantly constitute environmental nuisance.

Materials and Methods

Chemicals and Reagents

All reagents used for the current study where of analytical grade

Soil Sample Collection

A 3 m by 2 m area of the overflow region within the NPDC Oleh/Olomoro flow station was considered for this study. The site has over a decade presence of petroleum exploration activities which has left the environment with constant interface of pollution with petroleum and its allied products. This area was evaluated, cleared and grass chopping of the impacted area was done for easy access of the soil top layer. At the end of the clearing, the polluted area soil samples to the depth of 20 cm were collected into sterile polythene bags. Similar procedure was repeated for the non-polluted soil collected from a virgin land along Abraka-Aragba express way, Otorho-Abraka which served as the control. The choice of using Abraka as the control is based on the low pollution index reported earlier [7; 8].

Preparation Fruit waste and drying

Readily available putrefied orange, pineapple and water melon fruits and peels were taken from fruit sellers at Merogun waterside wasteland, Warri, Warri-South L.G.A. of Delta State. The putrefied fruits and peels were washed, sliced into minute sizes, and sundried for one week. After one week of sun drying, it was blended to form finely powdered dried fruit wastes (DFW). These were measured in a ratio of 30:30:40 % (orange, pineapple, watermelon) by weight. The originating fruit waste blend was then utilized for polluted soil amendment.

Design and Experimental Setup

The remediation process began by weighing the filtered soil sample (5 kg) per polythene planting bag. The soil sample was moistened to 60% water carrying capacity before distribution and treatment according to the following distribution.

Table 1: Experimental Set-up for Remediation Process

Groups	Soil Treatment		Post Amendment Analysis	
	20 % DFW	**40 % DFW**	**Week 1**	**Week 3**
Polluted Soil	-	-	Petroleum hydrocarbon,	Petroleum hydrocarbon,
T1-Ammended	+	-	physicochemical	physicochemical
T2-Amended	-	+	properties, enzyme,	properties, enzyme,
Control	-	-	microbial	microbial,

The presence of + and – signs indicates the presence and absence of a particular determinant.
KEY: DFW = Dried fruit waste

Unamended polluted Soil: 5 kg of contaminated Soil devoid of treatment.
Test 1: 5 Kg of contaminated Soils treated with 20 % weight by weight of the DFW mixture.
Test 2: 5 Kg of contaminated Soils amended by 40 % weight by weight of the DFW mixture.
Control: 5Kg of Soil from the selected control site devoid of contamination and treatment.
To boost aeration and even distribution of the ongoing remediation activities, the various samples of soil were turned every two days while soil sampling was performed at intervals of pre-remediation, week 1 and 3 weeks post remediation periods respectively.

Determination of Soil Physicochemical Properties
These were determined following already established standard protocols. Soil pH was by the use of pH meter with electrode as conducted by Bamgbose *et al.* [9]. Other parameters such as electrical conductivity (EC) employed methods described by Chopra and Kanzar [10], Soil phosphate [11], soil nitrate and chloride [12], for total organic carbon (TOC) [13].

Preparation of soil samples and metal (Cd, Pb, As, Cr, Ni, Mn, Fe, Cu) analysis
Soil preparation was carried out by the method of Csuros and Csuros, 2002 as reported by Osioma and Hamilton-Amachree [14]. Before adding aqua regia (1:3; HNO_3 and HCl), soil samples were ground up and put in a conical flask. Immediately after blending, 5 mL of perchloric acid was added, and a slid was used to cover it. The resulting mixture was then heated at 150 ^oC until it was decreased to approximately 5 mL. After cooling, filtering was carried out using Whatman No. 4 filter paper, which was then moved to a 100 mL volumetric flask and filled to the proper level with 1 M HNO_3.
The Atomic Absorption spectrometer was then used to analyze metal in triplicates. (MODEL 210VGP).

Analysis of Total Petroleum Hydrocarbon (TPH)
TPH concentration was conducted using modified methods of the United States Environmental Protection Agency (USEPA) (2000) by Gas chromatography-Mass spectrometry (GC-MS) as contained in standard laboratory procedure. The extraction process of the hydrocarbon content was carried out with solvent of 50:50 mix of acetone and dichloromethane (DCM) employing the cold extraction technique. Procedurally, 10 g aliquot sample was measured into a solvent rinsed beaker. Thereafter, 50 ml of the solvent mix (Acetone/DCM) was added to the sample. It was spiked with 1 ml of the surrogate mix. The sample was placed in the Sonicator for 10-15 minutes at about 700 ^oC. Ten grams of anhydrous sodium sulphate was added to the already sonicated sample until a clear extract developed and the extract poured into a round-bottom flask. The process was repeated on the residual soil with the additional 50 ml of the solvent mix (Acetone/DCM), sonicated and the beaker was allowed to settle and decanted into the same round-bottom flask. The beaker was then left to settle and decanted into the same round-bottom flask. Hexane was used to concentrate the solvent to 1:3 ml, and the concentrated liquid was then dispensed into a Teflon-lined vial with a capacity of 2 ml, suitable for TPH/PAH analysis. The HP Agilent 6890 Gas Chromatograph (Agilent Technologies, 610 Wharfdale Road, Wokingham, Berkshire, United Kingdom) was used to analyse the extracts. It is a computerized acquisition device with Agilent Chemistation software. As a transport gas, inert helium gas (2 ml/min) was used. For 1.50 minutes, the column was kept at 35 °C TPH actual figures were computed.

Preparation of Soil and assay for Enzyme activities
One hundred ml of phosphate buffer, pH 7.4 was added to 10 g of soil sample and stirred. The soil suspension was filtered using cheese cloth. The filtrates were then centrifuged at 7000 g for ten minutes to obtain supernatant (S_1). Soil enzyme activities were determined using standard

protocols with resulting supernatants as protein source. Based on this, the method of Rani *et al*. [15] was used to assay for soil catalase and peroxidase activities, soil dehydrogenate [16], soil amylase activity[17], Alkaline Phosphatase activity [18].

Determination soil microbial counts

Total Heterotrophic Bacterial (THB) Count

This was done using the pour plate technique and the procedure outlined by Dawoodi et al. [19] on nutrient agar (Oxoid). In a nutshell, nutrient agar of known weight (28 g) was combined with 1 litre of distilled water and left to percolate for ten minutes. After seeing it spin and mix, autoclaving was carried out for 15 minutes at 121 °C. When the mixture had cooled to about 47 °C, it was inoculated by adding 0.1 ml of serial dilutions to a sterile petri dish, followed promptly by warm nutrient agar. After solidification, the petri plates were turned over and left to incubate for 48 hours at room temperature (28±2 °C). To determine the number of bacteria per gram of soil, plates producing counts of 30 to 300 colonies were selected, and the counts obtained were multiplied by the dilution factor.

Hydrocarbon Utilizing Bacterial (HUB) Count

The Vapour-phase transfer method [20] was adopted in the estimation of the hydrocarbon utilising bacteria. The serial dilutions of the samples obtained from the soil samples were inoculated into modified mineral salt medium. The medium components comprised of Magnesium sulfate (0.42 g $MgS0_4 \cdot 7H_2O$) potassium chloride (0.297g of KCl), Potassium hydrogen orthophosphate (0.85 g KH_2PO_4), Sodium nitrate (0.424 g $NaNO_3$), dipotassium hydrogen phosphate (1.27 g K_2HPO_4), Sodium chloride (20.12 g NaCl) and 20 and 250 grams of Agar powder and Amphotericin B respectively. The media constituents after measuring were emptied into 1 litre distilled water contained in a conical flask. Upon sterilization of the petri dishes via autoclaving at 121 °C, with a pressure of 15 Psi for fifteen minutes, inoculation of the gelled mineral salt agar (MSA) was carried out using appropriate dilutions of the soil samples and a Whatman filter paper soaked with crude oil were aseptically placed onto the covers before covering and inversion of the petri dishes. The hydrocarbon saturated filter papers supplied hydrocarbon by vapour-phase transfer to the respective inoculums and were incubated at a temperature of 28 °C ± 2 °C for 7 days. Colony counts were counted from triplicates and mean values were recorded in colony forming units per g (Cfu/g).

Total Heterotrophic Fungal (THF) Count

Using the pour plating method [21], heterotrophic fungi were counted. This was accomplished by adding 0.1 ml of samples that had been serially reduced ten times to an acidified potato Dextrose agar that also contained Streptomycin (1 mg/100 ml). For three to five days, the inoculated potato dextrose Agar dishes were incubated at room temperature. Colony formation units per gram, which represent the number of observed colonies, were calculated ($Cfug^{-1}$).

Hydrocarbon Utilising Fungal (HUF) Count

After aseptically diluting each 1.0 g soil sample 10 times in nutrient broth, 0.1 ml aliquots of each sample were placed into three triplicate plates of Bushnell-Hass (mineral salt) agar that had been fortified with 0.05% (v/v) streptomycin. The vapour phase transfer method [20] was used to aseptically insert sterile Whatman filter papers soaked in Bonny light crude oil into the lids of each inoculated Bushnell-Haas agar plate in order to test for hydrocarbon-using fungi. The Bushnell-Haas agar was incubated at 30°C for 14 days following the inoculation protocols. Colony formation units per gram ($Cfug^{-1}$), which measure the number of colonies that were visible after incubation, were calculated and represented as the hydrocarbon-using fungi.

Statistical Analysis

All the results were expressed as means ± SD and all data were analysed using Analysis of variance (ANOVA). Post Hoc Multiple Comparison between the control, pre-remediation period and treatment means was determined at 5 % confidence level ($P<0.05$) using Bonferoni Test.

RESULTS AND DISCUSSION

Effect of DFW mixture on selected Soil Physicochemical Properties.

Table 1 presents the effect of DFW on selected physicochemical parameters following week 1 and 3 post amendment periods. Relatively, the pH, Electrical conductivity (EC), Phosphate, Chloride and Total organic carbon (TOC) were significantly ($P<0.05$) different from each other as can be seen across the experimental groups in Table 2. Taken together, the soil physicochemical properties is indicative that the soil amendment have impacted positively on the soil in a time dependent manner by contributing to the improvement of soil physicochemical properties of

the amended soil relative to the unamended polluted soil.

It is imperative to state that a soils physiochemical characteristic is the first indication of the soils wellbeing and remains the primary mechanism by which petroleum hydrocarbon pollution imparts on agricultural and plant products. This is so true because the rapid loss of microbial activities imparts directly on the availability of soil micro nutrients needed for the plants to grow (Oyem and Oyem, 2013) [2]. The observed reductions in the soil pH following the two periods of post amendments, may be related to the gradual increase in hydrogen ions owing to degradation of petroleum [21-23]. Earlier, Osuji and Nwoye [24] have given similar assertion in their study noting that petroleum degradation mediated decrease in pH is attributable to the increased concentration of organic acids in response to microbial metabolic activities in relation to petroleum and organic agents used for remediation.

Soil EC is known to determine the relative abundance and concentration of charged ions. The highly concentrated charged ions (cations and anions) in the oil-impacted locations may be the cause of the noticeably higher EC values found in post-amended soil samples. According to Benson et al., [25], one explanation for the amended soil's decreased nitrate content is that it acted as a source of nutrients for oil degraders. As previously demonstrated [26; 27], phosphorus is required for the creation of nucleic acids, cell membrane, and ATP. Nitrogen is necessary for the configuration of cellular proteins and cell walls.

The observations relative to low phosphate levels in polluted soils agrees with Osuji and Nwoye [24] and Gighi *et al.* [28]. According to [28], the low phosphate concentration of the contaminated soil was most likely brought on by the high contamination/pollution index (C/P) ratio as a consequence of the crude oil spill. Therefore, a sufficient supply of these elements is required for bioremediation of contaminated crude oil samples because these microorganisms need them for active growth and metabolic function [30]. The increase in the concentration of TOC within the duration of the study may have been due to microbial mineralization of the petroleum hydrocarbon and it is in agreement with the report of Obasi *et al* [31]. Also, the increase of TOC may be caused by the input of hydrocarbon-oil. This is however in order since hydrocarbons have high percentage of carbon. The decrease seen in the TOC of the amended treatments at 3 weeks post amendment periods could be a result of high carbon utilization by soil microorganisms as energy source for the oil degradation due to favourable pH level. Wyszkowski and Ziolkowska [32] reported that hydrocarbons presence in an agriculturally-fertile soil caused a reduction significantly in organic carbon and nitrogen concentration of the soil. Taken together across the soil physicochemical parameters, the findings of the present research show that DFW soil amendment at the end of 3 weeks was very effective in ameliorating the poor soil physicochemical parameters of the contaminated soils and this was both concentration and time-dependent.

Table 2: Effect of DFW mixture on Selected Soil Physiochemical Properties

Treatment	pH	EC (mS/m)	Nitrate(mg/kg)	Phosphate(mg/kg)	Cl(mg/kg)	TOC (mg/kg)
P_{US}	7.10±0.10^{a}	31.33±1.154^{a}	0.59±0.06^{a}	2.30±0.10^{a}	16.27±0.289^{a}	9.21±1.26^{a}
P_1-20	4.37±0.12^{b}	40.00±3.61^{b}	0.37±6.031^{a}	6.52±0.42^{b}	21.00±0.87^{b}	36.67±1.53^{b}
P_1-40	4.33±0.06^{b}	41.00±4.00^{b}	1.83±0.63^{a}	5.46±1.03^{b}	32.67±0.289^{c}	43.00±1.00^{c}
P_3-20	5.20±0.25^{c}	34.00±1.00^{a}	0.28±0.35^{a}	10.08±1.59^{c}	27.17±1.16^{d}	12.67±1.15^{a}
P_3-40	5.60±0.56^{c}	49.67±1.15^{c}	0.53±0.41^{a}	0.73±0.32^{d}	34.17±3.21^{c}	22.00±1.00^{b}
P_0	7.57±0.21^{a}	30.67±0.58^{a}	0.54±0.05^{a}	10.67±0.80^{c}	20.67±0.29^{ab}	11.67±1.53^{a}

All Values are expressed as Mean ± SD of three replicates. Values followed by different alphabets superscripts indicates that they differ significantly at $p<0.05$ While values with identical alphabet superscripts indicates that they are not statistically significant.

KEY: P_{US} = Un-amended Polluted Soil, P_1-20= 1 Week Post amendment 20%; P_1-40= 1 Week Post amendment 40%; **P_3-20**: 3 Weeks Post amendment 20%; P_3-40; 3 Weeks Post amendment 40%; P_0= Unpolluted Control Soil; EC =Electrical Conductivity; Cl = Chloride; TOC = Total Organic Carbon

Effect of DFW mixture treatment on deposit concentration of selected heavy metals in a crude oil polluted soil.

Significantly, the lead, copper, chromium, nickel, manganese, and iron concentration ($P<0.05$) was different from each other across the experimental groups as can be seen in Table 2. Thus in essence, the dried fruit waste was able to ameliorate the concentration of heavy metals in the polluted soils post amendment. This is in positive correlation with the study carried out [33] using agricultural wastes where there was reduced heavy metals concentration of Cobalt by 76%.

Table 3: Effect of dried waste fruits mixture treatment on deposit and concentration of selected heavy metals in a Crude oil Polluted Soil

	Lead (Pb)	Copper (Cu)	Chromium (Cr)	Nickel (Ni)	Arsenic (As)	Manganese (Mn)	Iron (Fe)	Cadmium (Cd)
P-$_{US}$	1.94±0.02^{a}	0.22±0.05^{a}	21.48±6.61^{a}	3.02±0.03^{a}	<0.003^{a}	1.23±0.11^{a}	51.82±1.48^{a}	0.18±0.02^{a}
P$_1$-20	0.26±0.07^{b}	0.21±0.012^{a}	16.36±0.39^{ab}	2.01±0.28^{b}	<0.003^{a}	1.07±0.04^{bd}	54.08±1.00^{a}	0.14±0.04^{a}
P$_1$-40	<0.010^{c}	0.16±0.012^{ab}	12.27±0.50^{bc}	2.35±0.08^{b}	<0.003^{a}	1.13±0.01^{ab}	51.24±1.10^{a}	0.12±0.02^{a}
P$_3$-20	<0.010^{c}	0.20±0.02^{ab}	11.26±0.91^{bc}	2.10±0.19^{b}	<0.003^{a}	0.67±0.02^{c}	30.48±6.14^{b}	0.13±0.05^{a}
P$_3$-40	<0.010^{c}	0.13±0.01^{b}	5.26±1.11^{dc}	0.95±0.01^{c}	<0.003^{a}	0.60±0.01^{c}	33.46±3.80^{b}	0.12±0.01^{a}
P$_0$	0.02±0.00^{d}	0.17±0.012^{ab}	9.01±2.25^{bd}	2.29±0.01^{b}	<0.003^{a}	0.93±0.02^{d}	33.81±1.08^{b}	0.12±0.01^{a}

All Values are expressed as Mean ± SD of three replicates. Values expressed by different alphabets superscripts indicates that they differ significantly at $p<0.05$ While values with identical alphabet superscripts indicates that they are not statistically significant.

KEY: P-$_{US}$ = Un-amended Polluted Soil, P_1-20= 1 Week Post amendment 20%; P_1-40=Soil; 1 Week Post amendment 40%; **P_3-20**: 3 Weeks Post amendment 20%; P_3-40; 3 Weeks Post amendment 40%; P_0= Unpolluted Control Soil;

Effect of DFW mixture treatment on total petroleum hydrocarbon.

The results expressed in Table 4 below shows the effect of the DFW amendment on the TPH after 1 week and 3 weeks amendment. Relatively, the TPH (both aliphatic and polycyclic aromatic) differ from each other as can be seen across the experimental groups in Table 3. Thus, dried waste fruit mixture had potential for ameliorating the hydrocarbon content in crude oil polluted soil. There was loss in TPH in both soil samples amended with DFW mixture significantly ($P<0.05$). The effects of time factor on petroleum degradation was significant in post 3 week's amendment in comparison to post 1week amendment polluted soil. With the rate of hydrocarbon breakdown increased with time and concentration, it also corresponded with the bacteria growth result. This may imply that the soil amendment improved bacteria load which in turn reflected in the hydrocarbon breakdown. These observations are in agreement with works reported by other authors [26; 33; 34].

Table 4: Effect of dried fruit waste on concentrations of petroleum hydrocarbon in crude oil polluted soil

Treatment	Aliphatic (mg/l)	PAH (mg/l)	TPH (mg/l)
P-$_{US}$	177.80±0.99^{a}	65.17±3.03^{a}	242.98±3.70^{a}
P$_1$-20	116.29±0.83^{b}	80.60±1.54^{b}	196.89±1.58^{b}
P$_1$-40	154.17±0.39^{c}	42.44±0.49^{c}	196.61±0.21^{b}
P$_3$-20	101.17±0.44^{d}	69.83±0.52^{d}	170.99±0.96^{c}
P$_3$-40	119.87±0.63^{e}	49.49±1.51^{e}	169.34±1.15^{c}
P$_0$	0.00±0.00^{f}	0.00±0.00^{f}	0.00±0.00^{d}

All Values are expressed as Mean ± SD of three replicates. Values expressed by different alphabets superscripts on the same column indicates that they differ significantly at $p<0.05$ While values with identical alphabet superscripts indicates that they are not statistically significant.

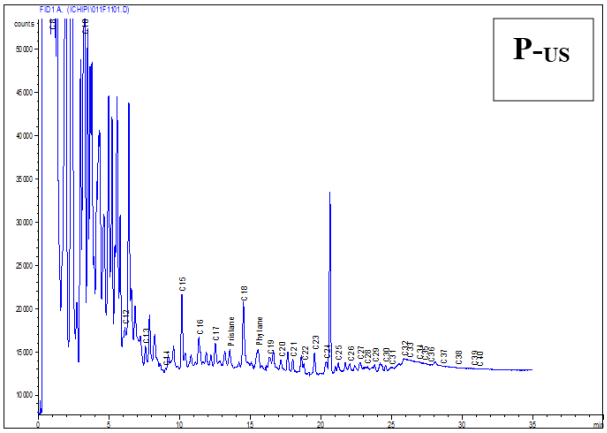
KEY: P-$_{US}$ = Un-amended Polluted Soil, P_1-20= 1 Week Post amendment 20%; P_1-40=Soil; 1 Week Post amendment 40%; **P_3-20**: 3 Weeks Post amendment 20%; P_3-40; 3 Weeks Post amendment 40%; P_0= Unpolluted Control Soil; **PAH** = Polyaromatic Hydrocarbon; **TPH** = Total Petroleum Hydrocarbon

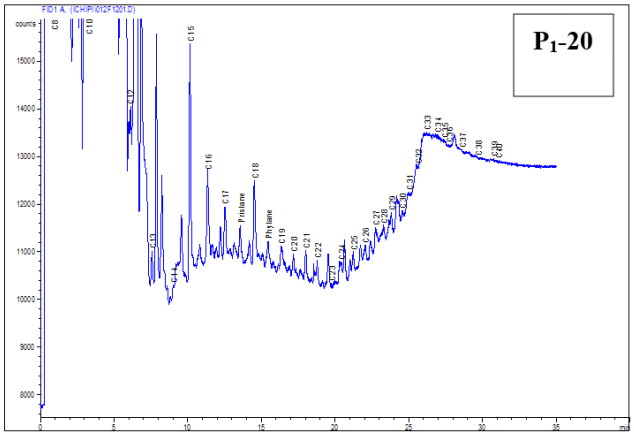
Gas Chromatography-Mass Spectrometry (GC-MS) Results:

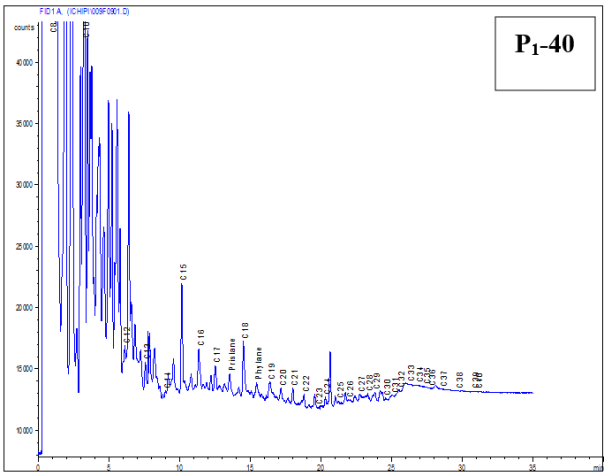
Identification of specific chemical compounds present in the crude oil soil samples was done following the method of the USEPA [37] using GC-MS analysis. The spectra of characterized pure chemical compounds in National Institute of Standards and Technology Library were used as

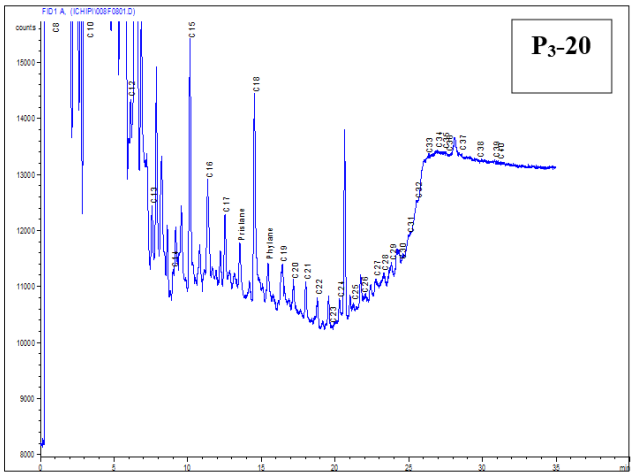
reference for identification of compounds present in the crude oil contaminated soil samples. The peaks are reflection of crude oil constituents present in the soil samples. All the peaks represent aliphatic compounds of different carbon length, likewise with that of polyaromatic compounds. According to [38; 39], aliphatic and aromatic compounds are toxic so are reflection of adverse effects on soil health and the environment at large.

According to [40], n-octadecane (C_{18}) and tetracosene (C_{24}) indicated in the polycyclic aromatic hydrocarbons on the spectra, are always associated with crude oil contamination and these compounds are said to lead to soil adverse effects and infertility due to their low solubility and high hydrophobicity hence their reduction is a sign of improvement in the soil health. The treatment of the soil mediated reductions significantly in the peaks as can be shown in the trend from about 30,000 relative abundance counts in the unamended polluted soil to about 13,000 counts after 3 weeks amendment with 40% w/w of DFW. The reduction may be due to the improved activities of soil microbes which may have been stimulated by the nutrients supplied by the DFW. The metabolism of toxic compounds such as the ones identified in the current study as a source of carbon supply have been previously reported [40], that n-Octadecane can be metabolized or degraded by Fusarium species (a fungus). The relative abundance (about 1500 counts) in the unpolluted control sample is infinitesimal compared to the contaminated soil samples across all experimental groups.









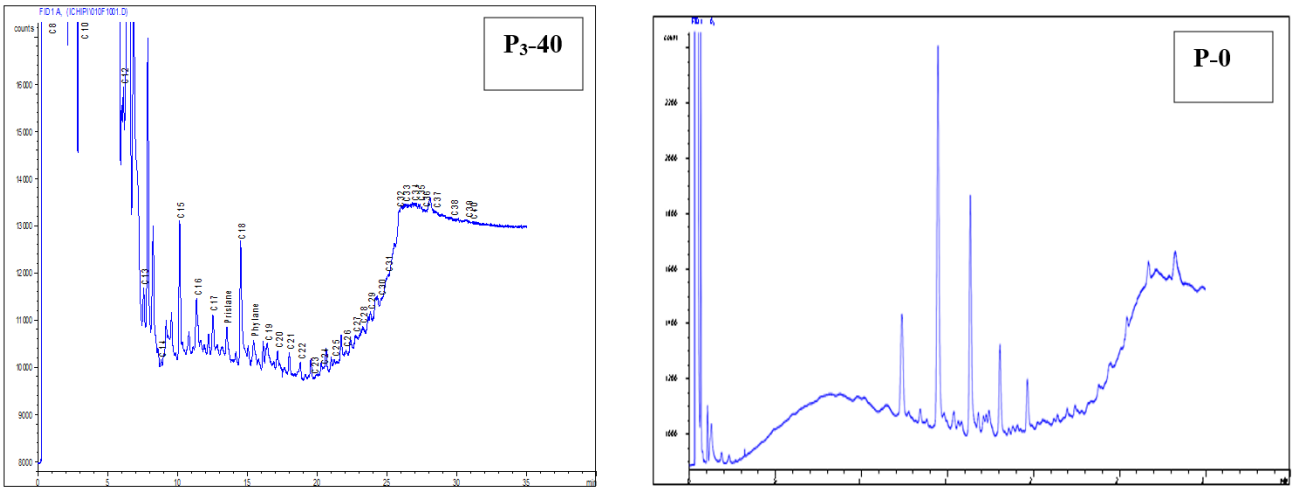
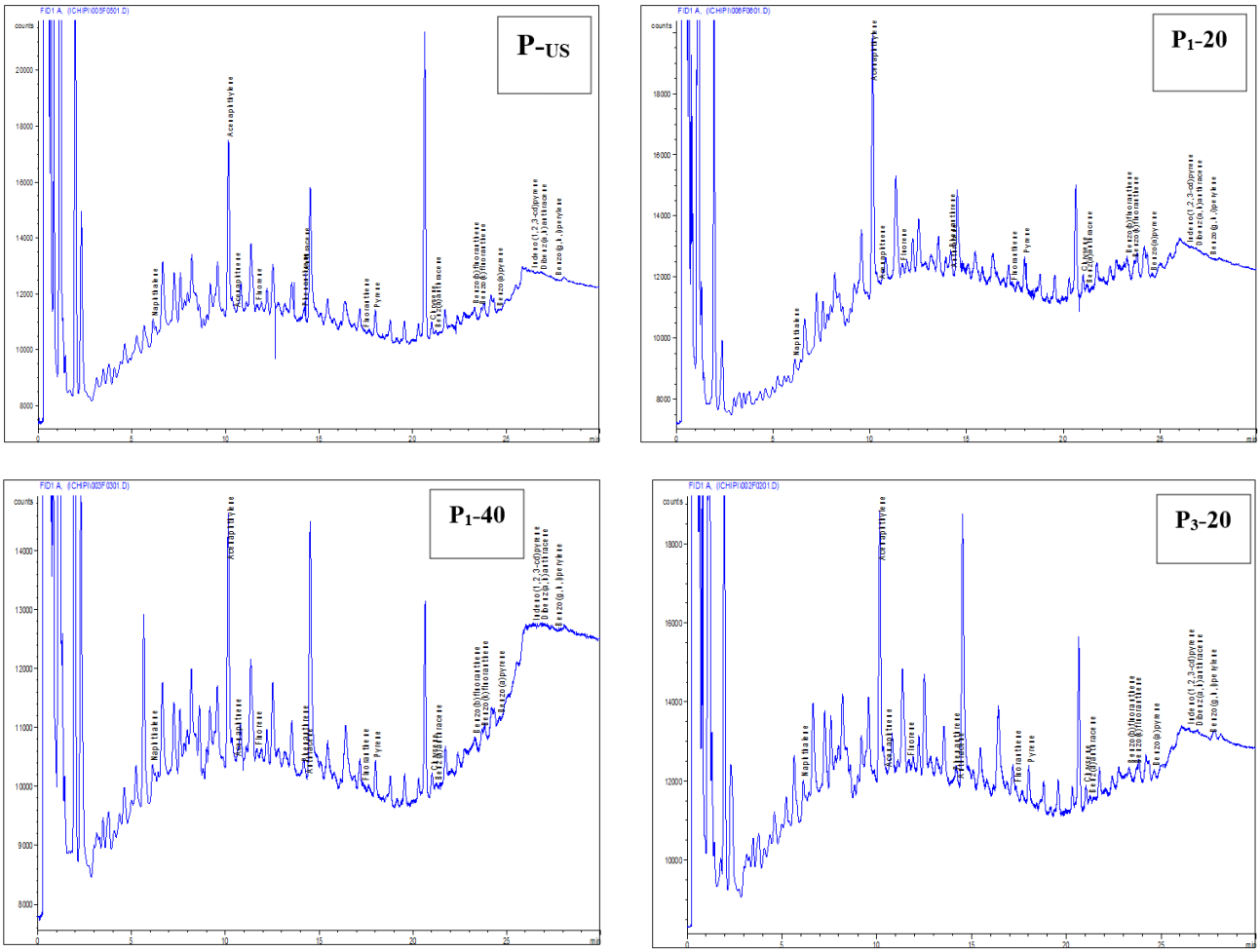


Fig. 1: The GC-MS Chromatogram of Aliphatic hydrocarbons of the soil samples

KEY: P-$_{US}$ = Un-amended Polluted Soil, P_1-20= 1 Week Post amendment 20%; P_1-40 =Soil; 1 Week Post amendment 40%; **P_3-20**: 3 Weeks Post amendment 20%; P_3-40; 3 Weeks Post amendment 40%; P_0= Unpolluted Control Soil



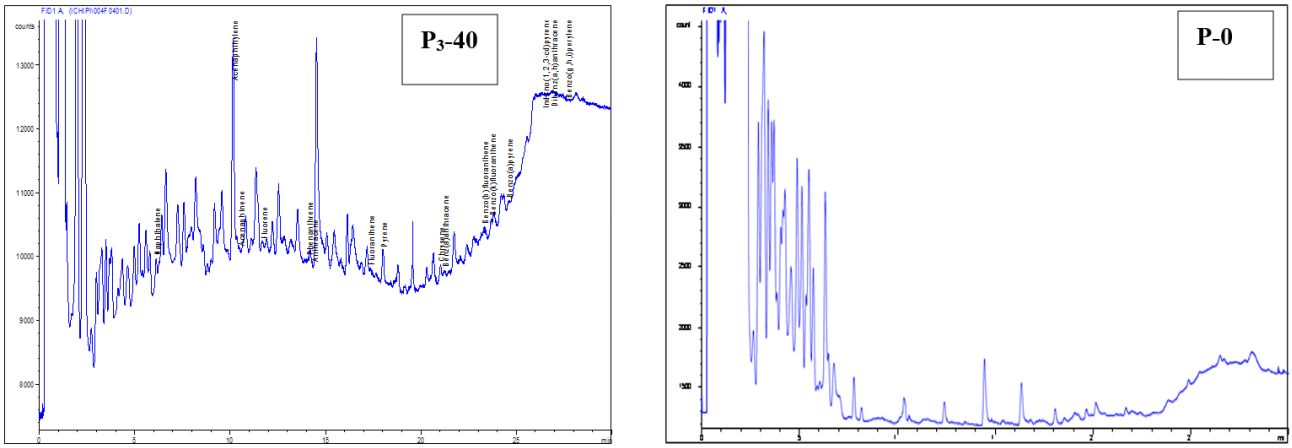


Fig. 2: The GC-MS Chromatogram of Polycyclic Aromatic hydrocarbons (PAHs) of the soil samples

KEY: P-$_{US}$ = Un-amended Polluted Soil, P_1-20= 1 Week Post amendment 20%; P_1-40 =Soil; 1 Week Post amendment 40%; **P_3-20**: 3 Weeks Post amendment 20%; P_3-40; 3 Weeks Post amendment 40%; P_0= Unpolluted Control Soil

Effect of DFW mixture treatment on the activities of selected soil enzymes (α-Amylase, peroxidase, alkaline phosphatase, soil dehydrogenase, and catalase) in a crude oil polluted soil.

The effects of the DFW amendment on the activities of soil enzymes after one week and three weeks are shown in the findings presented in Table 5 below. According to the results, the soil enzymes significantly ($P<0.05$) differ across experimental groups. Based on the aforementioned, the DFW mixture was effective in reversing the decline in the activities of the chosen soil enzymes in the crude oil-contaminated soils in comparison to the control soil sample. According to a 2017 study, [41] petroleum hydrocarbons change the biochemistry and enzymatic activity of soils. Achuba [42] also reported that hydrocarbon-oil contamination alters the way starch-metabolizing enzymes function and in turn, produces oxidative stress, which lowers the amount of amylase activity in soil significantly. The findings of Anigboro and Tonukari [43] during their study on the effects of crude oil on the activities of soil amylase and soil invertase in germinating cowpea seedlings and cassava leaf extract also support the decrease in amylase activity as observed in the crude oil contaminated soil relative to the control soil. Together, these decreases can be said to have been a reaction to the increased decline in soil nutrient mobilization. [44; 45].

After initially declining due to pollution in comparison to the control, amylase activity increased as the remediation process went on. According to [46], carbon and organic nitrogen sources are preferred for the synthesis of amylase, so the relative increase in soil amylase activity may be attributed to the DFW mixtures' potential for bioremediation as well as to better nutrient mobilization. The DFW combination can be considered an effective soil remediating agent in the presence of crude petroleum because the addition of glucose promotes crude-oil degradation [47; 48]. When compared to the non-polluted soil, the mean alkaline phosphatase activities in the polluted soil dropped non-significantly ($P>0.05$). The strong impact of petroleum hydrocarbon pollution on soil pH may have caused the observed rise in acid phosphatase activity. As a result, the environment's increased acidity makes the decreased pH favourable for enzyme activities [49]. While acid phosphatase is active in an acidic pH, alkaline phosphatase is more active in an alkaline pH [50]. The significant ($P<0.05$) rise in acid phosphatase activity in the DWF-remediated soil indicates that the hydrocarbons are still being broken down [51]. The non-significant variation in alkaline phosphatase activity might be due to the acidic environment inhibiting the enzyme. Significant changes in soil dehydrogenase activities were also noted, which may be explained by the elevated overall microbial respiratory rate. According to Yang et al. [48], the increased activity of dehydrogenases may be due to particular microbes using polyaromatic hydrocarbons, demonstrating the effectiveness of soil amendment conditions in reducing detrimental effects on soil enzymes.

Table 5: Effect of DFW blend on activities of selected soil enzyme in polluted soil

Treatment	Amylase (Unit/ml)	Dehydrogenase (µmol/g)	CAT (nmol/min)	Peroxidase (µmol/g/fw)	ALP (Unit/dl)
P-$_{US}$	0.63±0.09^{a}	19.99±0.14^{a}	2.88±0.53^{a}	1.62±0.22^{a}	1.58±0.31^{a}
P$_1$-20	0.60±0.13^{a}	16.94±1.74^{ab}	3.90±0.14^{a}	1.55±0.72^{a}	1.35±0.27^{a}
P$_1$-40	0.60±0.13^{a}	15.17±0.60^{b}	4.14±0.13^{a}	1.88±0.28^{a}	1.48±0.18^{a}
P$_3$-20	3.44±0.45^{b}	24.59±01.24^{c}	8.56±1.16^{c}	3.67±0.07^{b}	1.56±0.17^{a}
P$_3$-40	4.00±0.84^{b}	24.64±2.01^{c}	8.34±0.69^{c}	3.37±0.04^{b}	1.58±0.17^{a}
P$_0$	2.87±0.17^{b}	15.45±1.04^{b}	4.32±0.44^{a}	2.94±0.11^{b}	1.60±0.22^{a}

All Values are expressed as Mean ± SD of three replicates. Values expressed by different alphabets superscripts indicates that they differ significantly at $p<0.05$ While values with identical alphabet superscripts indicates that they are not statistically significant.

KEY: P-$_{US}$ = Un-amended Polluted Soil, P$_1$-20= 1 Week Post amendment 20%; P$_1$-40=Soil; 1 Week Post amendment 40%; **P$_3$-20**: 3 Weeks Post amendment 20%; P$_3$-40; 3 Weeks Post amendment 40%; P$_0$= Unpolluted Control Soil; **CAT =** Catalase; **ALP =** Alkaline phosphatase

Effect of DFW mixture treatment on the THB, HUB load, THF and HUF load in crude oil polluted soils

The results displayed in Table 6 shows the effect of DFW mixture treatment on the THB, HUB, THF and HUF dynamics in crude oil contaminated soils after 1 week and 3 weeks amendment. There is a difference significantly ($P<0.05$) in the THB count of all the experimental soil samples in comparison to the control as can be seen below. Therefore, taken in total across the bacterial and fungal in the soil, the findings of the present study show that the DFW mixture had the potential in ameliorating the polluted soils with a corresponding increment in the bacterial and fungal counts. The fast use of the hydrocarbon in crude oil by these bacteria as a source of carbon and energy may be the cause of the increase in the number of hydrocarbon-using bacteria. When the findings from the amended soils are compared to those from the control, it is clear that the DWF amendment was what caused the increase in the HUB counts. The results from the control also showed a substantial difference over time. According to earlier reports [52-54] the fact that certain oil-degrading microorganisms are present in large quantities in oil-polluted environments is proof that they are active environmental pollutant degraders. To sum up relative to the microbes in the soil, the findings of the present research show that the DWF had the potential in ameliorating the polluted soil with a corresponding increment in the population of indigenous oil degrading microbiota.

Table 6: Effect of DFW treatment on the microbial load dynamics in polluted soils

Treatment	THBx10^{4}cfu/g	HUB x10^{2}cfu/g	THF x10^{4}cfu/g	HUFx10^{2}cfu/g
P-$_{US}$	3.05±0.22^{a}	0.93±0.001^{a}	1.45±0.06^{a}	0.62±0.01^{a}
P$_1$-20	3.26±0.12^{a}	1.13±0.003^{a}	1.86±0.20^{a}	1.02±0.05^{b}
P$_1$-40	3.31±0.005^{a}	1.11±0.005^{a}	1.92±0.35^{ab}	1.08±0.003^{b}
P$_3$-20	3.48±0.07^{a}	1.22±0.02^{a}	2.22±0.27^{b}	1.46±0.02^{b}
P$_3$-40	3.08±0.015^{a}	1.49±0.03^{b}	2.44±0.15^{b}	1.52±0.01^{b}
P$_0$	2.18±0.01^{b}	0.320^{c}	1.08±0.01^{a}	0.0^{c}

All Values are expressed as Mean ± SD of three replicates. Values expressed by different alphabets superscripts indicates that they differ significantly at $p<0.05$ While values with identical alphabet superscripts indicates that they are not statistically significant.

KEY: P-$_{US}$ = Un-amended Polluted Soil, P$_1$-20= 1 Week Post amendment 20%; P$_1$-40=Soil; 1 Week Post amendment 40%; **P$_3$-20**: 3 Weeks Post amendment 20%; P$_3$-40; 3 Weeks Post amendment 40%; P$_0$= Unpolluted Control Soil; **THB**= Total Heterotrophic Bacteria (**X** 10^4 cfu/g**) HUB**= Hydrocarbon utilizing Bacteria **(X** 10^2 cfu/g**) TFC**= Total Fungi Count (**X** 10^4 cfu/g**) HUB**= Hydrocarbon utilizing Fungi (**X** 10^2 cfu/g**)**

Conclusion and Prospects for field trial

In this study, the nutrient content of fruit waste materials was improved by mixing different DFW (orange, pineapple and watermelon). The selection of the fruit waste as a constituent for the dried fruit mixture formulation was based on previous studies which has proven that their availability in large quantities and high nutrient content of either of

nitrogen, phosphorus or potassium can improve soil pH to favour microbial and plant growth. Based on the findings made in the current study, it was concluded that the utilization of a mixture of blended dried orange, pineapple and water melon fruit wastes contributed significantly to increased soil microbes population that were able to use available crude oil in the soils as carbon source which significantly contributed to the reduction of the total petroleum hydrocarbons in the crude oil impacted soils. The further reduction in the soils TPH eventually influenced in a positive direction soil physiochemical properties and soil enzyme activities. It is imperative therefore that this study which was conducted under a laboratory controlled environment can be replicated and explored on a field controlled trial. Under the field controlled trial, the growth and metabolic response of plants in crude oil remediated soils using the mixture of the studied fruit wastes can also be investigated. This is imperative because the field study if fully maximized has the potential of improving food production, promoting effective fruit waste management as well as contribute to several levels of job creation along the value chain.

Declaration of Competing Interest

The authors affirm that they have no known financial or interpersonal conflicts that might have looked to have an impact on the research presented in this paper.

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