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Evaluation Of Remediation Potentials Of Cassava And Orange Peels On Polycyclic Aromatic Hydrocarbons And Total Petroleum Hydrocarbons Contents In Crude Oil - Contaminated Soils

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Abstract
Crude oil pollution is a serious challenge in Nigeria especially in Niger Delta area. There is, therefore, a need to source an affordable method that is eco-friendly for remediating these polluted soils. The study investigated the use of cassava peel waste, orange peel waste, and mixture of cassava and orange peels as a bio stimulating agent in bioremediation of crude oil polluted soil. Treatments were as follows, NCS: Non contaminated soil to serve as Control, COS: Contaminated soil without treatment to serve as Control, CSC: Contaminated soil with cassava peel treatment, CSO: Contaminated soil with orange peels treatment, CSC+ CSO: Contaminated soil with mixed waste treatment. Total organic carbon, total petroleum hydrocarbon, and polycyclic aromatic hydrocarbon content, in soil were monitored at 0, 2, 4 and 6 weeks. Results showed that TPH and PAH in the soil after 4 weeks were as follows, CSC (2605.0 and 0.1100mg/kg), CSO (2429.2 and 0.2000mg/kg) and CSC+ CSO (1824.4 and 0.1667mg/kg), respectively. This result proves that cassava peel waste, orange peels and mixture of cassava and orange peels are effective bio-stimulating agent in crude oil degradation especially at the mixture at 4 weeks compared to other treatment options hence providing an alternative for the remediation of crude oil-polluted soil, especially in the Niger Delta area of Nigeria.

Keywords; *Remediation, Cassava, Orange Peels, Physicochemical, Crude Oil, Soils*

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I. Introduction

Soil is an important natural resource upon which environmental sustainability largely depends (Adenipekun, 2008; Onuh et al., 2008;). Several factors arising from natural and anthropogenic activities have rendered the soil impotent; since the soil is a repository of many wastes; thus, making the soil incapable of performing its natural endowed functions. In the Niger Delta region of Nigeria, one of such anthropogenic activities is crude oil exploration and exploitation by multinational oil companies. The invention of the internal combustion engine as a means of transport led to the increase use of crude oil as a source of energy (Chorom et al., 2010) with a concomitant increase in its production demand and transport (Difiglio, 2014).

These activities resulted in minor and major oil spillages into the environment which have caused severe damage to the ecosystem especially the soil. Apart from the spillages, the oil waste discharged into water bodies and soil from petroleum refining (Voulvoulis and George, 2015), also contribute significantly to oil pollution problem.

Due to the undesirable socio- economic and ecological consequences often associated with crude oil pollution (Panel, 2013); remediation of crude oil polluted soil becomes inevitable for good and efficient agricultural productivity, and proper and sustainable use of soil. Since natural attenuation of crude oil polluted soils may take a long time to accomplish and because of high demand for cultivated land, it may be inappropriate to allow polluted soils to be rehabilitated naturally. Several approaches have been adopted to decontaminate or restore a contaminated environment. Depending on the approach used, the remediation can be done either insitu or ex-situ. It is in-situ when the remediation is done on the polluted site and ex-situ when it is done outside the polluted site. Some of these approaches include physical/mechanical (burning, excavation, spray, vapor extraction, stabilization, solidification); chemical (detergent, surfactant), and biological (biostimulation and bioaugmentation) methods. The choice of method to be used depends on the circumstances and the suitability of the method in each case. Among the current approaches used for restoration or decontaminating crude oil polluted soils, bioremediation and chemical remediation appear to be taken the lead, although they have their limitations.

Bioremediation could be biostimulation (ie addition of nutrient to stimulate the biodegradation process) and biouagmentation (addition of biodegrading organisms). The rationale behind biostimulation is to remove nutrient limitations in soil usually associated with crude oil pollution. The chemical method involves the application of chemicals such as dispersant to dislodge the hydrocarbon chains and provide a good surface area for biodegradation (Couto et al., 2010). Though, the extensive application of this method has some reservations because of the fear of its toxicity and long-term environmental effect (USEPA, 1999).

The potential use of some organic wastes originating from plants such as rice husk and coconut shells, plantain peels, cocoa pod husk (Agbor *et al.* 2012) as biostimulating agents in the clean -up of soil contaminated with petroleum hydrocarbon have been investigated by some researchers. Similarly, cow dung, pig dung, poultry manure and goat dung all originating from animal wastes (Ijah & Antai. 2003) have all been reported. They were all found to show a positive influence on the petroleum hydrocarbon biodegradation in the contaminated environment.

Soya bean waste (SBW) was reported by (Ijah *et al.* 2003). From the microbiological analysis, the researchers found out that the aerobic heterotrophic bacteria count in the oil polluted soil amended with the soya bean waste were higher than that of the unamended contaminated or polluted soil with the following ranges: 1.6×10^8 cfu/g to 8.9×10^8cfu/g and 1.2×10^8 cfu/g to 3.6×10^8 cfu/g respectively. The extent of crude oil degradation in the polluted soil amended with 200 g SBW, 400 g SBW and the unamended soil were 54.6%, 70.4% and 8.8% respectively for 28 days.

The main routes for the entry and establishment of petroleum hydrocarbons (PHs) into the human body are from breathing ambient air and indoor air, PHs-contaminated food, smoothing cigarettes, breathing smoke from open fire places, etc., all causing a wide array of potential impacts on human health. For example, tobacco smoke contains benzo(a)pyrene (BaP) and more than 40 known or suspected human carcinogenic PHs. There are certain natural sources for releasing or holding PHs in the environment. Certain crops such as wheat, rye, lentils, etc. may synthesize PAHs or absorb PAHs from the environment (water, air, or soil).

Water may contain PHs and usually are leached into water from polluted soils or from industrial effluents and/or marine accidental spills. The best example for this is Ecuadorian Amazon streams wherein TPHs were in the range of 0.097–2.883 ppm, and these levels are nearly 10–288 times higher than the limit (0.01 ppm) set by European Community regulations. Moreover, the above levels of TPHs are even 10–10,000 times higher than the levels stipulated by the US EPA . Such higher levels of TPHs are attributed to intense petrochemical pollution caused due to the use of inadequate and outdated oil extraction methods by oil companies for >25 years during 1964–1990. In fact, BaP concentration in water was always lower in drinking water than untreated water, and it was about 100-fold lesser than the US EPA drinking water standard. According to the US EPA, 0.2 ppb of BaP in drinking water is considered as a maximum contaminant level (MCL).

II. Methodology

The Study Area

This experiment was carried out in a screen house located at the Teaching and Research Farm and the Soil Science Laboratory of Rivers State University (RSU) located at Port Harcourt. It lies between Latitude 4^0 46' N, and Longitude 7^0 10' E and Elevation of 13 meters above sea level. Port Harcourt is in the humid forest zone which has an average elevation of ten meters above sea level. The mean annual rainfall is 2400mm, usually in a monomodal distribution lasting from March to November. There is usually a dry spell between December and March with little or no rain, the wettest months are between July and October. Temperature varies from 27^OC from February to April (warmest months) to 25^OC in July and August (the coolest months). Relative humidity remains high throughout the year and varies from 78% in February (the driest month) to 89% in July and September (rainy months) (Ene, e*t al*., 2018).

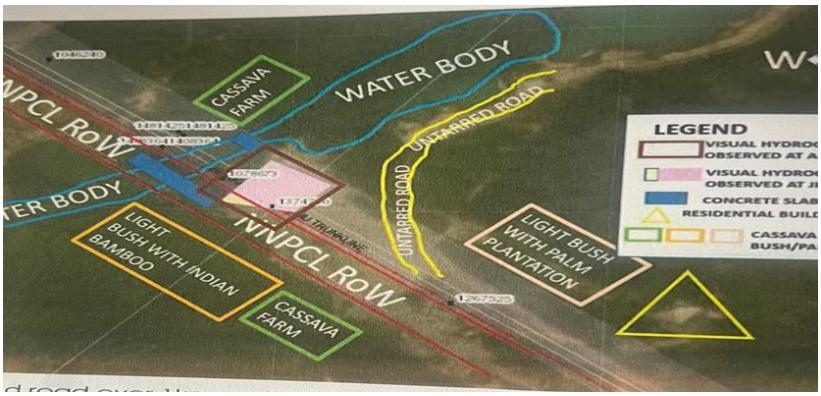

Fig. 1: Map of the Study Area

Collection of Soil Samples and Materials

Crude oil contaminated and uncontaminated soil used for the study were collected from the top surface soil (0 – 30cm) from Gio in Tai Local Government Area of Rivers State Fig. 1. The soil 0 – 30cm depth will be collected so as to cover the depth of penetration of the contaminant. 2kg of the soil was weighed and transferred into boxes of 40cm by 40cm dimension with the use of wooden boards.

Fresh peels of cassava tuber were collected from Abara in Etche LGA and orange fruit peels from orange sellers at Rumuokoro market in Obio-Akpor local government area of River State.

Treatment preparation

Cassava and orange peels were washed thoroughly with deionized water to remove sand and other impurities, sun-dried for 2 weeks and ground to powder using a grinding machine at mile 3 market. 5kg of each powdered peeled samples was weighed and mixed with the contaminated and uncontaminated soil.

These experimental boxes were moistened and properly homogenized before periodic sampling was carried out from each of the boxes at an interval of 14days for a total duration of 84days.

Experimental Design

The experimental design was a Complete Randomized Block Design (CRBD). These study involved 5 treatments each was replicated three times. Treatments were weighed and applied mixed in with the soil and allowed to sink gradually except for the unpolluted portion (0% crude oil) which serves as the control.

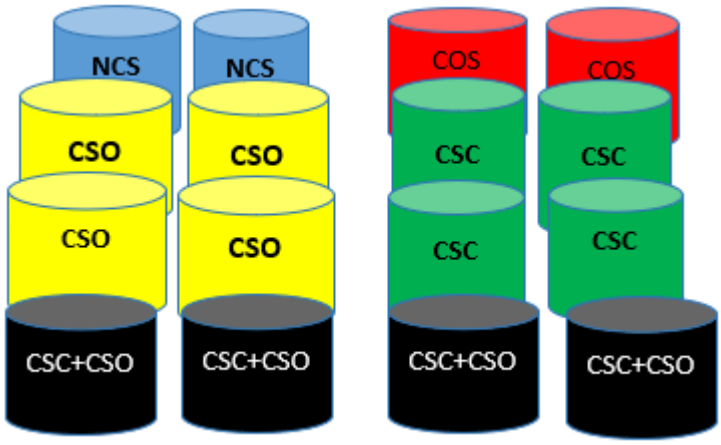

Fig 2: Treatment Layout

Where
NCS = Non contaminated soil to serve as Control
COS= Contaminated soil without treatment to serve as Control
CSC = Contaminated soil with cassava peel treatment
CSO = Contaminated soil with orange peels treatment
CSC+ CSO = Contaminated soil with mixed waste treatment

Analytical Methods

Soil Sample Extraction

Ten grams of soil sample was added into an amber glass bottle. Anhydrous sodium sulphate (Na_2SO_4) was also added into the glass bottle containing the soil sample. The sample was stirred. The addition of Na_2SO_4 was to remove moisture from the soil sample. 300 µg/ml of surrogate (1-chlorooctadecane) standard was added to the soil sample. 30 ml of dichloromethane (DCM) was added to the sample as extracting solvent and the bottle containing soil sample was corked very tight and transferred to a mechanical shaker.

The samples were agitated between 5 to 6 h at room temperature using a mechanical shaker. After agitation, the sample was allowed to settle for 1 h and then filtered through 110 mm filter paper into a clean beaker. The filtrate was allowed to concentrate to 1 ml by evaporation overnight in a fume cupboard.

Sample Clean-up

Sample clean-up was performed using glass column. Column preparation was carried out by inserting glass cotton into the column. Silica gel was dissolved with DCM to form slurry, and the slurry was added into the column. Anhydrous Na_2SO_4 was added into the column followed by addition of pentane. After preparation of the

column, the concentrated sample extract was mixed with cyclohexane in a beaker and transferred into prepared column. The sample extract was eluted using pentane as solvent and eluted sample collected in a beaker below the column. The sample was eluted further by adding more pentane into the column. After elution the column was rinsed with DCM. The eluted sample was allowed to stand overnight at room temperature in a fume cupboard for evaporation to take place.

Sample Separation and Detection

The separation and detection of compounds in soil samples was carried out using Agilent 6890N Gas Chromatograph - Flame Ionization Detector (GC-FID) instrument (Cortes et al. 2012). 3µl of concentrated sample eluted from column was injected into GC vial. The blank DCM was injected into micro-syringe of GC to clean the syringe (3 times) before taking the sample for analysis. The micro-syringe was further rinsed with the sample. Then the sample wase injected into the column for separation of compounds in the sample. After separation the compounds was passed through a flame ionization detector. FID detects the compounds in the sample. The amount of TPH and PAH will be resolved at a particular chromatogram in mg/kg.

III. Results And Discussion

The result for Polyaromatic hydrocarbon (PAH) and Total Petroleum Hydrocarbon (TPH) of soil treated with (CSO, CSC and CSO+CSC) and the contaminated control (COS) are shown in Figs. 2 and 3 respectively. Reductions in PAH and TPH were observed in polluted soil treated with orange, cassava peel and the combination (CSO, CSC and CSO+CSC) than in the control (COS). The highest reduction was recorded in polluted soil treated with combined peels (CSO+CSC) while the least was observed in the control (COS) at 2, 4 and 6 weeks. There was a significant difference (p = 0.05) in hydrocarbon reduction between treated soil and the control at 2, 4 and 6 weeks. There was also a significant difference (p = 0.05) in hydrocarbon reduction between treated soils at 2, 4 and 6 weeks as shown in Table 1.

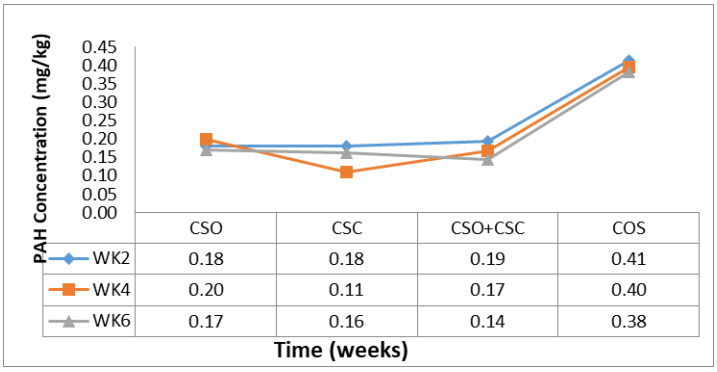

Fig. 2. Concentration of Polyaromatic hydrocarbon in the Treated Soils

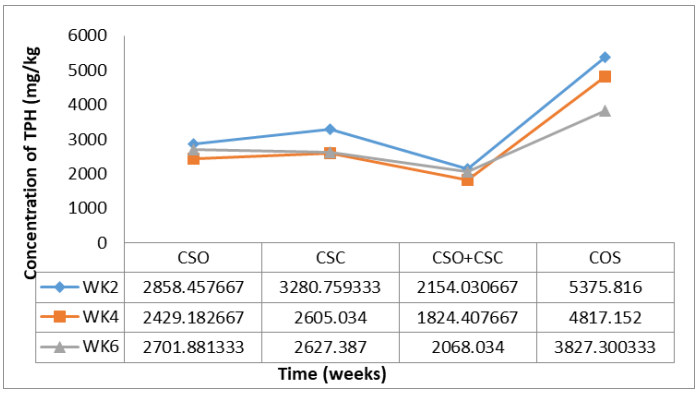

Fig. 3. Concentration of Total Petroleum Hydrocarbon in the Treated Soils

Table 1. Concentration of PAH and TPH in the soil

	PAH			**TPH**		
	WK2	WK4	WK6	WK2	WK4	WK6
CSC	0.1800^{b}	0.1100^{c}	0.1633^{b}	3280.8^{b}	2605.0^{b}	2627.4^{ab}
CSO	0.1800^{b}	0.2000^{c}	0.1700^{b}	2858.5^{b}	2429.2^{b}	2701.9^{ab}
CSC+CSO	0.1933^{b}	0.1667^{b}	0.1433^{b}	2154.0^{b}	1824.4^{b}	2068.0^{b}
COS	0.4833^{a}	0.3960^{a}	0.3833^{a}	5375.8^{a}	4817.3^{a}	3827.3^{a}

Table 2. Polycyclic Aromatic Hydrocarbon Concentrations

	PAH	CSO			CSC+CSO			CSC			COS		
		WK2	WK4	WK6	WK2	WK4	WK6	WK2	WK4	WK6	WK2	WK4	WK6
1	Naphthalene	0.01	0.01	0.02	0.01	0.02	ND	0.01	ND	0.01	0.02	0.02	0.02
2	Acenaphthylene	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.09	0.09	0.09
3	Acenaphthene	0.01	0.04	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.08	0.08	0.08
4	Fluorene	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.04	0.05	0.03	0.03
5	Phenanthrene	0.02	0.04	0.01	0.02	0.01	0.02	0.02	0.01	0.01	0.07	0.05	0.05
6	Anthracene	0.04	0.03	0.02	0.04	0.02	0.02	0.04	0.02	0.02	0.06	0.05	0.04
7	Fluoranthene	0.03	0.01	0.01	0.03	0.01	0.01	0.03	0.01	0.01	0.02	0.02	0.02
8	Pyrene	0.01	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.02	ND	ND	ND
9	Benz(a)anthracene	0.01	0.01	0.02	0.01	0.02	0.01	0.01	ND	0.02	0.04	0.02	0.02
10	Chrysene	ND	ND	ND	0.01	ND	ND	ND	ND	ND	ND	ND	ND
11	Benzo(b)fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	Benzo(k)fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.01	0.01
13	Benzo(a)pyrene	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.01	0.01
14	Indeno(1,2,3-c,d)pyrene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	Dibenz(a,h)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.01	0.01	0.01
16	Benzo(g,h,i)perylene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Total (ppm)	**0.18**	**0.2**	**0.17**	**0.19**	**0.16**	**0.14**	**0.18**	**0.11**	**0.16**	**0.48**	**0.39**	**0.38**

Table 2 shows the PAH content of the treated and untreated soil, in the treated soils with the cassava, orange and the mixture of both cassava and orange peels do not record the presence of the carcinogenic component of PAH, except the polluted control soil. According to United States Environmental Protection Agency, (USEPA, 2008), PAHs are classified as carcinogenic to animals. Meanwhile, some PAH-rich mixtures are also classified as carcinogenic to humans. The USEPA has classified the following seven PAH compounds: benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(ah)anthracene, and indeno(1,2,3-cd) pyrene as probable human carcinogens (USEPA, 2008).

Table 3. Total Petroleum Hydrocarbon Concentrations in Soil

TPH		CSC			COS			CSC+CSO			COS		
		WK2	WK4	WK6	WK2	WK4	WK6	WK2	WK4	WK6	WK2	WK4	WK6
PARAMETERS													
Aliphatic Components (ppm)													
n-Nonane (C9)		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-Decane (C10)		ND	ND	ND	ND	ND	ND	ND	ND	ND	0.025	0.025	ND
n-Undecane (C11)		ND	ND	ND	ND	ND	ND	ND	ND	ND	5.388	5.388	ND
n-Dodecane (C12)		ND	1.191	0.63	ND	0.014	ND	3.191	ND	ND	14.477	14.477	ND
n-Tridecane (C13)		1.801	9.823	9.2732	9.737477	2.595	1.27909	7.116	1.185	1.185	38.628	11.628	1.801
n-Tetradecane (C14)		17.396	40.716	40.1662	17.7813	28.868	10.75273	26.009	4.837	4.837	105.132	87.132	17.396
n-Pentadecane (C15)		23.4184	61.793	61.2432	44.75872	57.158	31.20841	47.086	15.288	25.288	135.204	117.204	43.839
n-Hexadecne (C16)		50.8994	81.694	81.1442	64.84207	86.133	48.03448	66.987	33.468	43.468	115.953	97.953	71.32
n-Heptadecane (C17)		40.9344	89.343	88.7932	69.62223	133.702	60.1489	75.636	47.676	57.676	265.47	247.47	61.355
Pristane		85.2104	269.118	268.5682	135.4382	217.719	126.8686	234.411	73.999	83.999	472.389	454.389	105.631
n-Octadecane (C18)		53.8604	146.982	146.4322	247.376	101.449	71.13783	132.275	53.992	63.992	266.197	248.197	74.281
Phytane		118.6424	205.369	204.8192	147.6069	199.682	154.1559	187.662	105.456	115.456	359.089	341.089	139.063
n-Nonadecane (C19)		26.1614	131.415	130.8652	190.2488	148.708	113.0808	104.708	95.876	105.876	243.382	225.382	46.582
n-Eicosane (C20)		71.5924	117.022	116.4722	128.634	84.046	77.843	102.315	105.099	115.099	164.576	146.576	92.013
n-Heneicosane (C21)		133.0684	153.832	153.2822	193.893	159.814	151.922	89.125	49.625	59.625	171.947	153.947	153.489
n-Docosane (C22)		81.0894	100.294	99.7442	144.903	133.001	95.214	85.587	47.258	57.258	216.507	198.507	101.51
n-Tricisane (C23)		79.7074	74.412	73.8622	56.999	101.22	67.799	59.705	78.498	88.498	116.052	98.052	100.128
n-Tetracosane (C24)		118.2214	120.659	120.1092	83.412	96.97	94.291	105.952	72.697	82.697	231.664	213.664	138.642
n-Pentacosane (C25)		159.6974	154.749	154.1992	108.667	123.474	179.188	140.042	124.661	134.661	288.981	270.981	180.118
n-Hexacosane (C26)		154.5264	92.075	91.5252	139.612	89.284	143.24	75.368	68.857	78.857	180.181	162.181	174.947
n-Heptacosane (C27)		125.7524	54.269	53.7192	86.334	51.231	52.634	39.562	105.559	115.559	255.403	237.403	146.173
n-Octacosane (C28)		51.2774	68.501	67.9512	46.148	66.971	147.114	53.794	76.584	86.584	162.048	144.048	71.698
n-Nonacosane (C29)		62.4284	65.248	64.6982	71.383	58.257	70.375	50.541	66.008	76.008	159.709	141.709	82.849
n-Triacontane (C30)		167.6394	118.494	117.9442	76.84	100.694	152.934	103.787	124.337	134.337	267.344	249.344	188.06
n-Hentriacontane (C31)		277.2364	206.357	205.8072	124.8	193.257	332.131	191.65	251.813	261.813	489.1	471.1	297.657
n-Dotriacontane (C32)		61.9924	32.877	32.3272	228.13	31.498	49.717	18.17	40.19	50.19	96.292	78.292	82.413
n-Tritriacontane(C33)		153.7764	95.931	95.3812	58.397	35.375	80.165	78.224	56.473	66.473	197.486	179.486	174.197
n-Tetratriacontane (C34)		85.0354	15.357	14.8072	142.304	42.944	37.22	0.65	19.459	29.459	125.512	107.512	105.456
n-Pentatriacontane (C35)		360.0944	52.123	51.5732	84.64	41.624	30.906	36.416	15.033	25.033	57.054	39.054	380.515
n-Hexatriacontane (C36)		109.3034	34.877	34.3272	36.495	29.772	37.032	20.17	11.693	21.693	83.665	65.665	129.724
n-Heptatriacontane (C37)		199.6354	6.302	5.7522	42.631	4.544	115.742	3.595	45.301	55.301	10.256	10.256	220.056
n-Octatriacontane(C38)		150.1924	4.488	3.9382	28.379	3.591	33.07	1.781	6.629	16.629	36.407	9.407	170.613
n-Nonatriacontane (C39)		19.4644	ND	ND	57.475	ND	96.848	ND	18.001	28.001	ND	ND	39.885
n-Tetracontane (C40)		240.7484	ND	ND	ND	ND	45.187	ND	0.889	0.614	ND	ND	261.169
Total TPH (ppm)		**3280.8**	**2605**	**2627.4**	**2858.5**	**2429.2**	**2701.9**	**2154**	**1824.4**	**2068**	**5375.8**	**4817.3**	**3827.3**

Results shown in Table 3 for Total Petroleum Hydrocarbon (TPH) level concentration in the different treatments, crude oil contaminated (COS) control recorded a higher range (3827.3 to 5875.8mg/kg) while the mixture of cassava and orange peels recorded the least with the range of (1824 to 2154mg/kg). from the Table 3, was observed the carcinogenic component of TPH according to IARC (International Agency for Research on Cancer) which are the Benzene and benzo(a)pyrene are not found in both control and the treated soils

Nutrient addition (cassava and orange peel) in this study, as bio stimulating agent to crude oil-polluted soil in the bioremediation process helped in speedy reduction of hydrocarbon of the soil to as low as a reasonably practicable condition.

High reduction in Total Petroleum Hydrocarbon (TPH) and Polyaromatic Hydrocarbon (PAH) observed in treated polluted soil against the polluted soil without treatments could be attributed to the addition of orange, cassava and combination of orange and cassava peels to polluted soil. The peels acted as a bio-stimulating agent accelerating the biodegradation of the hydrocarbon contaminant. This finding agrees with Jude, et al (2022), which observes a corresponding decrease in the hydrocarbon contents when cassava peels, was used as a bio stimulant in remediation of crude oil polluted soil. The rapid degradation of hydrocarbon observed in the treated soil could also be because both the orange and cassava peels added to the polluted soil stimulated the catabolic process of indigenous microorganisms thus enhancing the utilization of the contaminant as a source of energy and carbon (Ijah and Antai, 2003, Andreolli, *et al.*, 2015). Agbor, *et al*. (2018). Reported cassava peel as an agricultural waste with the potential for enhancement of reduction of crude oil in polluted soil. Ideriah, et al, (2023) also reported on the potential of cassava and orange peels on crude oil contaminated soil. Similar observations have been reported using other organic amendments (Al-Kindi, and Abed, 2016). It has been earlier reported that crude oil-polluted soil amended with the organic matter may stimulate the growth of the indigenous oil-degrading microbiota in it (Coulon, and Delille, 2003, Abioye, et al., 2010.). from the findings, cassava and orange peels can completely remediate the soil from carcinogenic component of PAH.

IV. Conclusion

From the result of this study, it could be concluded that the biodegradation of petroleum hydrocarbon was significantly enhanced by the addition of cassava peel, orange peels and the combination of cassava and orange peels to the crude oil polluted soil as higher hydrocarbon (TPH and PAH) reductions and remediation of hydrocarbon mostly occurs at the fourth week These results demonstrate that mixing cassava peels, orange peels with contaminated soil, under selected solid-culture conditions, is a good alternative for soil bioremediation. The usage of cassava peel, orange peels and mixture of both in the bioremediation of crude oil-polluted soil is hereby recommended. Remediation of crude oil contaminated soils should be done for four week

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