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Crude Oil Contamination Effect on The Rhizosphere Soil Properties of *Zea mays L.* and *Vigna unguiculata* (*L.*) Walp.

Onyiba, P. O[1], Asa, A. A[2], and Okoh, T[3]

[1, 2, 3] National Root Crop Research Institute, Umudike Abia State,
Federal Ministry of Agriculture and Rural Development, Nigeria
princewillogugua@yahoo.com

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Abstract

Crude oil contamination poses a significant threat to soil ecosystems, impacting agricultural productivity and crop growth. This study investigated the effects of crude oil contamination on the rhizosphere soil properties of maize (*Zea mays*) and cowpea (*Vigna unguiculata*), two economically important crops, grown in a potted sandy loam soil. Through a series of controlled experiments which were simply arranged in a completely randomised design with four replicates, and four levels of crude oil contamination (0%, 0.5%, 2% and 5% v/w), with each replication containing eight treatments. The control samples were not contaminated. The presence of crude oil in the rhizosphere soil led to significant altercations in microbial diversity and activity, potentially affecting nutrient cycling and overall soil health. These microbial organisms were isolated at ten days interval, which include *Nitrosomonas, Nitrobacter, Rhizobium, Azotobacter, Bacillus, Pseudomonas, Aspergillus, Penicillum, Fusarium, Clostridium, Micrococcus, Enterococcus and Arthrobacter.* It was also observed that *Bacillus spp, Pseudomonas spp, Micrococcus spp*, *Clostridium spp* persisted in the soil after crude oil contamination. Hence, they have potential for degrading petroleum products. Also, the population of petroleum degrading bacteria among the two crops were significantly increased, which ranges from 2.00 to 9.40 x 10^8 Cfu/g in maize, while 0.50 to 4.90 x 10^8 Cfu/g in cowpea. As the crude oil contamination rates increases, the growth of nitrifying bacteria, nitrogen fixing bacteria, and fungi were significantly affected in both soil grown with maize and cowpea crop. The results demonstrated a substantial and significant decline in soil pH, nutrient levels (nitrogen, phosphorus and potassium levels), organic matter content, exchangeable bases and acids in the crude oil-contaminated rhizosphere soil compared to uncontaminated soil. Additionally, increased levels of heavy metals were observed in the contaminated soil, indicating a potential toxic impact on crop growth and soil microbial communities. Overall, this study underscored the detrimental effects of crude oil contamination on the rhizosphere soil properties of maize and cowpea, emphasizing the importance of remediation strategies and sustainable agricultural practices to mitigate the environmental consequences of oil pollution on crop production and soil health. Further research is highly needed to explore long-term impacts and develop innovative solutions to address the challenges posed by crude oil contamination in agricultural systems.

Key words: Crude oil contamination; Rhizosphere Soil; Soil Properties; Maize; Cowpea; Microbial diversity

Introduction

Crude oil contamination poses significant threat to terrestrial ecosystems, including agricultural lands in Nigeria and the world at large. Crude oil, a complex mixture can adversely impact soil quality, ecosystem depletion, biodiversity, nutrient availability and crop growth. Crude oil and its refining products consist of aliphatic, oleic, naphthenic and polycyclic aromatic hydrocarbons, which can change the physical and chemical characteristics of soil and its structure. These hydrocarbon compounds which are harmfully obnoxious and toxic in nature are largely responsible for changed fertility of agricultural soils. The affected contaminated soils lose their physical, chemical and biological activities and can require some reasonable amount of periods to recover and reclaim the soil fertility (Wyszkowska *et al*, 2001). Eze *et al* (2013) noted that one of the ways soils loss their biological activities, which was caused as a result of crude oil contamination is the reduction or inhibition of microbial activities. Affected Soils differ from the uncontaminated virgin soils due to changes in their physicochemical properties as well as biological properties (Robertson *et al*, 2007). If a soil is contaminated with hydrocarbon compounds, there occurs a change in organic carbon, and biological properties leading to an initial reduction/inhibition of soil microorganisms especially in soils that have not been previously contaminated. Hofman *et al* 2004 recorded that though there are increases in number of soil microorganisms in PHC-polluted soils, as time goes on different species of organisms often decrease/increase over time. However, this reduction of organisms is rapidly followed with an increase in the population of microorganisms that are capable of degrading the soil contaminants (Seglers *et al*, 2003). Hydrocarbon compounds may interfere with the mutual relationship of plant-fungus relationships by changing the soil environment in such a way that movement of diffusible chemical signals (auxins) is prevented. Toxicity of hydrocarbon compounds in the soils containing humus and microorganisms have been found to be less severing.

It was observed that some soil microorganisms may appear resistant to crude oil hydrocarbon leading to degrading of these compounds and as a result benefit from the presence of these contaminants. He also noted that some fungi may have the ability to degrade these contaminants which they utilise as the source of energy and food for their survival and growth. Microorganisms of particular interest in this research study are rhizo-bacterial flora (such as nitrifying bacteria, nitrogen fixing bacteria), petroleum degrading bacteria and fungi, and this occurs due to their many beneficial roles in the soil environment. These organisms are beneficially important for the degradation of pollutants, bio-fertilization through nitrogen fixation, phyto-stimulation and bio-control of soil-borne plant diseases (Seglers *et al*, 2003).

The region of soil around the roots of plants is simply known as the rhizosphere. The rhizosphere is noted to contain a higher populations and greater diversity of microorganisms than soil with no plant. This is because plant roots exude chemical compounds into the soil that increase microbial activities by giving nutrients to the organisms. These chemical exudates consist of enzymes, aliphatic, aromatics, amino acids, sugars and low molecular weight carbohydrates (Robertson *et al*, 2007).

Soils contaminated with crude oil harmfully affect crop production (Bamidele, 2010; Lin *et al*, 2009). The two crops used in this research work include maize, *Zea mays* L.; and cowpea, *Vigna unguiculata* (L.) Walp, *which* are staple crops used for food and livestock feeds in different sections of Nigeria. Among the crops, the leguminous crops (cowpea) play a vital role in the restoration and sustenance of soil fertility through their ability to fix atmospheric nitrogen in partnership with certain rhizobacterial species (such as rhizobium spp). Cowpea (*Vigna unguiculata* (L.) *Walp*.) is a main source of dietary protein which can complement staple low-protein cereals and tuber crops (Singh *et al*, 2002; Langyinto *et al*, 2003). It is also used as a cover crop to curtail weeds in the agricultural land (Valenzuela *et al*, 2002). Cowpea plays a significant role in feeding animals during the dry season. Maize (*Zea mays*) is one of the major staple cereal crop planted for food, feed and industrial purposes (USAID, 2010; Oyewo, 2011). Maize is also a key source of carbohydrate and vitamins in human food. A useful quality of vitamins C and A can be derived from white and yellow grains respectively. For industrial purposes, maize grain can be used as livestock feeds and also serve as a raw material for starch, flour and alcohol production (Agoda *et al*, 2011). Therefore, this trial was to assess the effect of crude oil contamination on the rhizosphere Soil of *Zea Mays L*. and *Vigna Unguiculata* (L.) Walp, with regard to the chemical properties and microbial dynamics of the rooting region of planted soil.

Material and Method

Experimental Location: The experimental study was conducted in the Department of crop and soil science, Faculty of Agriculture, Rivers State University, Port Harcourt, Nigeria in a controlled environment.

Experimental Design: A completely randomised design (CRD) as the study was a 4 x 2 factorial experiment, with each treatment replicated four times to ensure statistical robustnes. The factors comprised of four(4) levels of crude oil contamination (0%, 0.5%, 2% and 5%) and two test crops (Maize and cowpea), with each replication containing eight (8) experimental units/pots totalling thirty-two pots.

Sources of Crude Oil: The crude oil samples were obtained fresh from the production plant of Nigeria National Petroleum Cooperation(NNPC) Port Harcourt Refinery, Alesa-Eleme, Rivers state, Nigeria. Sandy loam soil was obtained from the Teaching and Research farm of Rivers State University, Port-Harcourt. The soil sampled had no previous exposure to crude oil contamination.

Planting Materials/ Seed Viability: The viable seeds of *Zea mays* and *Vigna unguiculata* used in the experiment were sourced from the gene bank section of International Institute of Tropical Agriculture(IITA), Ibadan and Rivers State Agricultural Development Programme (ADP) and stored at a room temperature(25-30^{o}C) for 24hrs. Seed Viability tests of the seeds were conducted to ensure uniformity and viability of seeds.

Soil Preparation and Treatment: Rhizosphere soil was collected from the study site and were air-dried, sieved and dispensed in 5kg weights into thirty-two (32) plastic bucket perforated at the bases. The buckets were grouped into four replications of eight each, and each group was used for the two of the crop plants. Each plastic bucket, containing the rhizosphere soil samples, in a group was contaminated and mixed with one of four different levels of light crude oil (0%, 0.5%, 2.0% and 5% v/w). All control samples were without crude oil contamination, but were planted. All buckets with soil samples and crude oil were allowed for a week before planting. Thereafter, viable seeds of the crops were planted in the buckets and watered every three days by spraying. The experiment lasted for a months, and was repeated twice.

Data Collection: Data collected were soil PH (Electrode method)**,** Organic carbon (Black, 2000), Available phosphorus, Total Nitrogen (modified kjeldahl method**)**, Heavy metal content (Atomic Absorption Spectrophotometer), Exchangeable bases such Ca, Mg, Na, K (titration method and flame photometer) and Exchangeable acids (titrimetric method).

Determination of Microbial Components: Microbial samples were collected from rhizosphere soil of both *zea mays* and *Vigna unguiculata* crops. Microbial components such as Nitrifying bacteria, Heterotrophic bacteria, Nitrogen fixing bacteria Hydrocarbon-degrading bacteria, and Fungi were analyzed using standard and acceptable agar microbiological procedure .Microbial population count was also determined as follows;

Total no of Colonies/gram of soil = Number of colonies/Dilution Factor x Amount plated.

Statistical Analysis: Research data obtained were simply subjected to analysis of variance (ANOVA) to determine significant treatment groups, while the Significant means were separated with least significant difference (LSD) at 5% confidence level ($p<0.05$).

Results

Table.1 Chemical properties of unpolluted and polluted rhizosphere soil planted with the two crops

Soil Properties	Before Pollution	Maize (% v/w)				Cowpea(%v/w)			
		0	0.5	2	5	0	0.5	2	5
PH	6.17	6.11	6.21	6.23	6.26	6.12	6.16	6.25	6.26
Organic. C (%)	1.84	1.58	2.52	2.91	4.70	1.81	2.33	2.71	4.20
T.Nitrogen (%)	0.134	0.09	0.16	0.13	0.17	0.06	0.11	0.13	0.19
Avail. P (mg/kg)	69.93	67.76	53.52	52.2	51.12	64.43	56.67	54.9	49.33
Exch. Bases(mol/kg									
Ca	2.27	2.31	2.51	2.72	2.53	2.57	2.41	2.61	2.21
Mg	1.20	1.16	1.15	1.21	1.11	1.11	1.12	1.21	1.01
Na	0.06	0.05	0.04	0.06	0.05	0.06	0.04	0.05	0.05
K	0.08	0.08	0.07	0.06	0.06	0.08	0.07	0.07	0.06
Exch . Acidity	1.56	1.52	1.71	1.42	1.61	1.45	1.61	1.77	1.41
Heavy Metals									
Fe	923.5	1042	1051	1122	1262	1043	1214	1252	1314
Cu	18.20	20.3	19.36	22.5	24.3	20.3	18.5	19.36	20.86
Zn	67.55	82.2	85.6	82.7	85.76	82.7	84.8	80.76	87.6
Mn	253.60	373.73	47.12	371.2	389.7	353.2	360.8	363.6	361.6
Pb	4.90	5.01	5.02	5.01	5.07	5.01	5.05	5.04	4.89
Cd		1.61	1.46	1.22	0.86	1.06	0.9	1.22	0.56

Maize, Cowpea ; $LSD_{0.05}$ for pH = ns, ns; Organic carbon = 2.42 , 0.76 : Available Phosphorus= 2.20 , 1.89 ; Total Nitrogen= 0.20 , 0.18; Exchangeable Acids= 1.08, 0.44 ; Iron (Fe)= 4.12, 3.98; Copper (Cu)= 1.72, 0.60 ; Lead (Pb) = 0.32, 0.18;

Crude oil contamination and rhizospheric soil chemical properties. The rhizospheric soil chemical properties in the experimental pot were presented in Table.1. The soil was an acidic sandy loamy soil with no previous hydrocarbon content, but had originally have soil pH of 6.17, nitrogen 0.134%, avail. Phosphorus 69.93 mg/kg and organic carbon 1.84%. Upon introduction of crude oil compounds, the soil pH was not significantly affected, and organic carbon content of the rhizosphere soil increased significantly while available phosphorus reduced to 51.12 and 49.33mg/kg at 5% soil treatment for maize and cowpea respectively. Furthermore, total nitrogen content was slightly increased at 5% to 0.17 and 0.19% for maize and cowpea respectively, 4 weeks after planting.

The soil pH was 6.17 slightly lower than the soil pH at the conclusion of research for the different treatments. At 0.5% to 5% rhizosphere treated soils, the soil pH was slightly increased to 6.26 for maize and 6.26 for cowpea compared to the soil pH of uncontaminated soil. The increased levels of crude oil contamination also increased the organic carbon content of the rhizosphere soils of maize to 4.70, and of cowpea to 4.20 compared to the uncontaminated soils which was 1.84. Available phosphorus decreased with the application of increased crude oil compared to the result of the control. The effect of crude oil treatments on available phosphorus of the rhizosphere soil of the test crops was significantly ($P<0.05$) different. Available phosphorus of the planted soil also reduced to 51.12 for maize and 49.33 for cowpea. There were also significant reductions on the exchangeable bases (sodium, potassium and magnesium) in the planted soil of each crops compared to the uncontaminated soil.

The details of the heavy metal concentrations in both the contaminated and uncontaminated rhizosphere soil of the crops were also shown in Table.1. Crude oil contamination significantly ($p<0.05$) affected the individual elements of heavy metal concentration in the soil planted with the two crops. The table showed that high concentrations of lead and cadmium were observed in contaminated soil of maize, 5.07 and 1.61

while low concentrations were observed in polluted soil of cowpea, 4.98 and 0.56. Higher concentrations of iron were observed in polluted soil of maize, 1262 as compared to unpolluted soil, 923.5. Similar trend was observed in cowpea with concentration of 1314. Higher concentrations of manganese were recorded in contaminated soil of maize, 389.7 while the lowest concentrations of manganese were observed in uncontaminated soil of the crops, 253.60. Similarly highest concentrations of manganese were recorded in contaminated soil of cowpea, 361.6. Highest concentrations of zinc were also observed in contaminated soil of maize and cowpea, 85.76 and 87.6 whereas the lower concentrations were observed in uncontaminated soil of the crops, 67.55. Also, higher concentrations of copper were observed in contaminated soil of maize and cowpea, 24.3 and 20.86 respectively.

Table 2: Effect of crude Oil Contamination on Microbial Population of the Crops

Crop/Treatment	Maize					Cowpea				
	C	**0**	**0.5**	**2**	**5**	**C**	**0**	**0.5**	**2**	**5**
10^8Cfu/g										
NFB	5.75	5.65	3.43	2.43	1.50	3.25	2.90	1.20	0.45	3.85
NB	8.78	8.70	5.53	3.90	3.53	4.90	3.50	2.34	1.50	4.50
THB	6.35	6.25	3.80	2.20	1.64	4.50	2.30	2.00	1.21	5.80
PDB	2.00	2.00	4.00	7.10	9.40	0.50	1.50	4.00	4.90	1.50
10^7Cfu/g										
F	6.80	5.70	3.60	2.12	2.00	7.25	6.05	4.80	2.50	6.01

NF : Maize, and Cowpea, LSD=1.38, and 1.33 ; NB: Maize, Cowpea LSD=0.60, 1.30 ; THB: Maize, Cowpea , LSD=1.17, 1.21 ; PDB: Maize, Cowpea , LSD=1.41, 1.32 ; F: Maize, Cowpea , LSD=1.07, 1.67 ;
LSD= Least significance difference at 5% confidence level ($p<0.05$); C= control; NFB= Nitrogen fixing bacteria; NB= Nitrifying bacteria; THB= Total heterotrophic bacteria; PDB = Petroleum degrading bacteria; F= Fungi; Cfu/g= Colony forming unit per gram.

Table 3. Microorganism isolated from Crude oil contaminated and uncontaminated soil.

Nitrifying Bacteria	Nitrogen-fixing Bacteria	Fungi	Petroleum degrading Bacteria	Heterotrophic Bacteria
Nitrosomonas euroaea	*Azotobacter nigricns*	*Aspergillus flavus*	*Clostridium Pasteurianum*	*Bacillus pumilus*
		Aspergillus niger		*pseudomonas mallci*
Nitrobacter vulgaris	*Rhizobium phaseoli*		*C. botilinum*	
		Aspergillus fumigatus		
			Bacillus Polymyxa	*Enterococcus feacalis*
	Rhizobium leguminosarium	*Aspergillus aclad*		
			B. circus	
		Penicillum citrinum		
	Bacillus Polymyxa		*B. megatorium*	
		Pencillum trequentum		
	Pseudomonas aeruginosa		*B.pumilus*	
		Fusarium roseums		
		Tricoderm horizonum	*Pseudomonas aeruginosa*	
		Cephalosporium sp	*P. avriginosa*	
			Micrococcus luteus.	
			Arthrobacter	
			Acinetobacter	
			Flavobacterium	

Crude Oil contamination and microbial Diversity of the rhizosphere soil. Microorganisms of the rhizosphere soil in oil contaminated and uncontaminated soil were presented in Table 2 and Table 3. The table 3 revealed the isolated microorganisms from crude oil contaminated and uncontaminated soil, whereas Table 4 showed the sensitivity of crude oil microbial degraders. Crude oil had a significant effect ($p<0.05$) on the microbial population of the rhizosphere soil of the two crops. There were significant ($p<0.05$) decrease in microbial count in the soil of maize and cowpea with 5 % levels of contamination. For instance, the nitrifying bacteria and nitrogen fixing bacteria counts from the soil of two crops were significantly reduced to 3.53×10^8 cfu/g and $1.50 \text{ x} 10^8$ cfu/g for maize, and to $1.50\text{x}10^8$ and $0.45 \text{ x}10^8$ cfu/g for cowpea compared to control of the respective crops. The population densities of the nitrogen fixing bacteria were ranged from 5.75 to 0.30×10^8 cfu/g while the densities of the organism in the soil of maize were from 5.75 to 1.5×10^8 cfu/g. The same trend happened in the soil of cowpea which was from $3.25 \text{ x } 10^8$cfu/g to $0.45 \text{ x } 10^8$ cfu/g. The rhizosphere of maize and cowpea exposed to 0 % to 0.5% (v/w) contaminations recorded the least observable population densities of hydrocarbon degrading bacteria whereas 5% contamination showed the highest result. The results also showed that the initial bacterial densities of 2.00×10^8 cfu/g and 0.50×10^8cfu/g recorded for maize and *cowpea* in uncontaminated soil when exposed to crude oil contamination increased, with time, to 9.40×10^8, 4.90×10^8 cfu/g, and 7.0×10^8. The hydrocarbon bacterial counts obtained for legume were 0.50×10^8 cfu/g. The density of the oil degraders in the plants rhizosphere increased overtime, even in soils with the high level (5%) of contamination. In contrast, the uncontaminated soil had lower densities of crude oil degraders. Mean density of 0.50 to 4.90×10^8 cfu/g was recorded for cowpea. Inversely, it was also observed that the microbial populations of total heterotrophic bacteria were lower in crude oil contaminated rhizosphere soil of the test crops. The total heterotrophic bacteria were 1.64×10^8 cfu/g and 1.21×10^8 cfu/g lower in the contaminated soil than in the uncontaminated rhizosphere soil. Fungi counts in the rhizosphere of cereal and legume crops in soil contaminated were also presented in Table 2. The rhizosphere of cowpea was observed to enhance greater fungal growth, 2.50×10^7 cfu/g compared to that of maize, 2.00×10^7 cfu/g at 5% crude oil contamination. There were, however, a significant ($p<0.05$) decrease in fungal growth on the rhizosphere of the test crops caused by the crude oil contamination.

Discussion

Crude Oil contamination and microbial Diversity of the rhizosphere soil. A significant difference ($P\leq0.05$) was observed between the effects of the crude oil on the rhizomicrobial flora and uncontaminated soil flora of the test crops. The microbial communities in the rhizosphere of the cereal and legume crops in the crude oil contaminated soil were observed as it is seen in Table 2. The crude oil greatly affected soil bacterial and fungal species of these test crops. The results indicated that hydrocarbon compounds had a significant effect on the total heterotrophic bacteria counts. It was also observed that there was significant decrease at the 5% level of the crude oil contamination for these organisms due to the prevailing unfavourable conditions created by the hydrocarbon compounds which might have reduced the microbial population of total heterotrophic bacterial. Li *et al*, 2007 reported that there were reductions in the heterotrophic bacteria activity in a crude oil-contaminated soils with decrease in the functions of their soil enzymes such as polyphenol oxidase, and dehydrogenase. Table 2 revealed the microorganisms isolated from the rhizosphere of maize and cowpea planted in crude oil contaminated and uncontaminated soil (control). Though the crude oil affected some of these organisms which reduce and/or disappear during the course of the research, most organisms were able to tolerate and survive the harsh conditions of crude oil contaminations. Table 5 also indicated that some organisms could utilize and degrade hydrocarbon compounds which allow them to tolerate the toxicity of contaminants. These microbial agents which can survive high toxicity of crude oil contaminated soil are called degraders as they utilized hydrocarbon compounds as a source of energy and food for their survival, growth and multiplications. They have the capacity to degrade the crude oil contaminants and revitalise the soil. The microbial communities of the crude oil contaminations in the test crops increased overtime, even in soils with the high level (5%) of contaminations. However, the uncontaminated soil had lower microbial populations of crude oil degraders.

Mean populations of 2.00 to 9.40 x 10^8 cfu/g and 0.50 to 4.90 × 10^8 cfu/g were observed for maize and cowpea due to the presence of hydrocarbon compounds which serves as source of energy for the hydrocarbon degrading bacteria. With this development, it favoured the rapid multiplications of hydrocarbon degrading bacteria, thereby resulting in high population of the organisms in contaminated soil. The lower microbial populations of hydrocarbon degrading bacteria observed in uncontaminated rhizospere(control) soil were due to non-availability of hydrocarbon compounds in the soil. Moreover, it was observed that gradually hydrocarbon degrading bacteria increased in some of the treatments of the test crops. The increase was a result of the available crude oil which crude oil bio-degraders utilised for energy and carbon source. It was noted that there was an increase in the multiplications of microbial communities in a crude oil contaminated soils. In the same vein, it reported that plant rhizosphere are highly favourable for the proliferations, multiplications and metabolism of microbial because of the plant chemical exudates that are released to the soil, hence the multiplication of rhizobacteria populations as observed, with increase in quantity of nutrient accumulations in the soil. In addition, gradual increases in soil minerals and nutrients have simply improved microbial growths due to availability of nitrogen, sulphur, carbon and energy(Chikere *et al*, 2003). Nutrients such as nitrogen, sulphur and carbon are essentially important in the synthesis of amino acid in the microbial growth.

Table 4: Screen Test for Utilisation of petroleum Hydrocarbon by Bacterial Isolates

Isolate Codes	Growth in Crude Oil	Bacterial Isolates
A_1	++	*Flavobacterium sp*
B_1	++	*Micrococcuss spp*
B_2	++	*Bacillus Ceresus*
A_2	+++	*Pseudomonas aeruginosa*
		Arthrobacter spp
A_3	+	*Arthrobacter*
C_1	+	
		Acinetobacter Spp
D_1	++	
		Clostridium pasteurianum
C_2	++	*Bacillus polymyxa*
D_2	++	*Azotobacter sp.*
D_3	++	

NB: +++ = Heavy Growth; ++ = Moderate growth; + = little Growth.

In the Table 4, the results revealed that the rhizospheres of maize and cowpea grown in contaminated and uncontaminated soil harbour diverse species of microorganisms. Further, higher populations of nitrogen-fixing bacteria were found in the rhizosphere soil of legume cultured in uncontaminated soils. The increase in the levels of contaminations resulted in decrease in the multiplication of nitrogen fixers and nitrifiers with time. Expectedly, nitrogen-fixing bacteria were found in the rhizosphere of the cowpea. Cowpea exposed to the high (5%) level of contaminations harboured the least population of nitrogen fixers. The uncontaminated soil supported the highest counts of 5.65 and 3.25 x 10^8 cfu/g for maize and cowpea respectively. The results showed that crude oil generally affected the replications, survival, growth and multiplications of nitrogen fixers in the test crops. Moreover, nitrifying bacteria were seen to be sensitive to the crude oil levels that even 2% contaminations were significantly different compared to the control. This reduced with increased concentrations of crude oil in the rhizosphere soil of the test crops. Again, under the influence of crude oil contaminations, nitrifying bacteria could not effectively thrive with other microorganisms that grow and multiply rapidly, leading to reductions in the available inorganic nitrogen.

It was noted that aerobic nitrogen fixers relatively multiply abundantly than other microorganisms while nitrifying organisms considerably reduced in population. In addition, Muratova *et al* (2003) reported that soil contaminated with organic compounds such as bitumen reduced the population counts of denitrifying, amonifying, nitrifying, nitrogen fixing bacteria in the rhizosphere soil of plants like reed and alfalfa.

Table 4 showed that fungal populations were observed to reduce with increase in the concentrations of crude oil. At 0.5, 2% and 5% contaminations, the population counts of fungi in maize rhizosphere were lower than those in cowpea. Though both rhizosphere soils of the crops received the same crude oil treatments, the chemical exudates from the cowpea roots must have ameliorated the effect of the crude oil contaminations, thus showing increase in microbial counts of fungi in its rhizosphere compared to those of the maize. Ekpo *et al* (2007) observed that the microbial populations in the rhizosphere soil were substantially different in different root regions and that a microbial community in the rhizosphere may be changed by alterations in root chemical exudates caused by changes in plant nutritional qualities.

The isolated bacterial species such as *Bacillus pumilus, Pseudomonas mallci, Enterococcus feacalis, Micrococcus luteus* and all fungal species persisted after the crude oil contaminations. This could be due to the fact that these microorganisms have the ability to synthesize and degrade the crude oil, thereby improving the nutritional status of the soil for their growth and survival. Yong *et al*, (2006) stated that in a crude oil contaminated soil, some bacteria and most fungi have inherent enhanced physiological tolerance and the ability to utilise the crude oil compounds. Some microorganisms, for example *Clostridium botulinum, Listeria monocytogen and Rhizobium leguminosarium* were cultured and isolated only before contaminations. These organisms could have been eliminated because they were able to make use of hydrocarbons as their sole source of carbon and energy (Avidano *et al*, 2005).

The screen test for the crude oil degrading ability of the bacterial isolates show the strong hydrocarbon degrading potential of *Clostridium pasteurianum, Bacillus polymyxa, Azotobacter* sp and *Pseudomonas aeruginosa* within the first 10 days of exposure to the crude oilcontaminations. Though *Clostridium pasteurianum, Bacillus polymyxa and Pseudomonas aeruginosa* maintained their hydrocarbon degrading abilities under prolong contamination to crude oil in soil, most of the nitrogen fixing bacteria including strong bio-degraders such as *Azotobacter* species, together with *Nitrosomonas* and *Nitrobacter* with moderate hydrocarbon degrading potential seemed to have lost their degrees of degradability. Milic *et al*, (2009) observed that Pseudomonas sp. and Bacillus sp. were found in crude oil contaminated soil, whereas reductions occurred in the total microbial populations due to the accumulations of petroleum waste sludge. Hydrocarbon degraders have the ability to tolerate oil contaminated soils because they have the capacity to utilize hydrocarbons as their source of energy (Katsivela *et al*, (2005). In addition, it was confirmed that petroleum waste sludge adversely affected the microbial communities by decreasing essential inorganic nutrients and growth factors, and reducing the pH immediately around negatively charged soil surfaces. Obviously, only certain nitrogen fixing bacteria have the ability to grow on nitrogen free media or very low in nitrogen sources. *Bacillus polymyxa, Azotobacter* sp, *Clostridium pasteurianum* and *Pseudomonas aeruginosa*, as seen in the research work, could grow heavily on crude oil concentrations using them as sole source of carbon and energy (Chibuike *et al*, 2013).

Crude oil contaminations and rhizospheric soil chemical properties. Crude oil, undoubtedly, had significant effects on some chemical properties of the soil. Organic carbon, for example, increased significantly ($p<0.05$) as the crude oil levels increase. This increase in crude oil levels occurred as a result of the introduction of crude oil into the soil. As the organic carbon increased under normal circumstances, it was expected to reduce the soil fertility and quality. This is because the increase in organic carbon was crude oil-associated, it rather reduces the soil quality and fertility. It would be observed that the crude oil sealed up soil pore spaces, and as a result. Prevent water the infiltration and movement and air into the soil, thereby reducing the biological activities and disrupting biochemical conversions necessary to make essential nutrients and some minerals available to the crop plants. Soil pH was not significantly affected by the oil. Reduced soil pH, increases in soil organic carbon and organic matter, sodium, iron were observed in crude oil contaminated soils (Obire *et al*, 2002). Okoro *et al*, (2005) observed that soil sodium and iron increased in crude oil-contaminated soils. Crude oil had a inhibitory effect on the macro and micro nutrient levels in the soil, with the exclusion of organic carbon which increased as the crude oil was progressively introduced. There were significant reductions ($P<0.05$) in the levels of soil sodium, potassium, manganese and calcium with the introduction of crude oil levels, when compared to their levels in the uncontaminated control. Soils contaminated with crude oil contain heavy elements such as iron, copper, zinc, manganese, cadmium, lead and so on (Table 4). The Table 4 showed that high concentrations of heavy metals were observed in all contaminated rhizosphere soils. Among all the heavy metals detected in the soil, high concentration of lead were observed in contaminated soil of maize,5.07 while the low concentrations of lead were observed in that of Cowpea,4.89 compared to the control. This is a strong prove that maize could serve as hyper-extractor which could be used in the process of phytoremediation of crude oil contaminated soils. These heavy elements could oxidize and form a harmful compound. Heavy elements could form coordinate bonds and complexes with ligands such as ammonia, water and nitrogen oxide or with other elements such as potassium, calcium, magnesium etc. When such complexes and bonds happened, the bound ions lost their ionic properties and will be undetectable in solution. Available phosphorus was significantly ($p<0.05$) reduced as the crude oil levels increased (Osuji *et al*, 2007). kayode *et al,(*2009) reported that crude oil contaminations decreased the levels of soil nitrate and phosphorus but the effects on other macronutrients remained investigated. A research work conducted by Wyszkowsk *et al*, (2001) on the crude-oil contaminated soils reported that there were an increase in the levels of nitrogen, phosphorus and potassium as the soil was amended with inorganic fertilizers. Some of these unessential mineral nutrients like calcium, magnesium and phosphorous are required for seed germination and plant growth. However, this ability of maize to tolerate and withstand the toxicity of hydrocarbon compounds and grow in concentrations that cause death of other crops makes it a possible candidate for the phytoremediation of crude oil contaminated soils. This is because for any crop to be used in the bioremediation process, it must possess the capacity to germinate and grow in the hydrocarbon contaminated soil. Kayode *et al*, (2009) defined phyto-remediation as the process by which biological techniques, with respect to the use of plants and micro-organisms, are used for the purpose to remediate contaminated soil and water.

Conclusion

Microbial activities at the rhizosphere soil of the test crops were inhibited, with the exception of hydrocarbon degraders, as the concentrations of the crude oil contaminant increased. The results showed that crude oil contaminations of soil at low concentrations, 0.5 to 2% enhance microbial multiplications and growth in sandy loam soil, which contained cowpea crops while high concentrations lead to growth inhibition in maize and cowpea. It was also observed that high concentrations of crude oil favour the growth and the survival of crude oil degraders such as *Bacillus sp, Pseudomonas spp, clostridium sp, Arthrobacter sp* etc. The results gotten from soil chemical analysis implied that crude oil created adverse and unfavourable condition to the soil health status and composition which made hydrocarbon contaminated soils unfit for farming activities. The results also showed that crude oil contaminations have unbearable impacts on the soil pH, mineral nutrients like nitrogen, phosphorus and potassium, organic carbon content, and microbial dynamics of soils contaminated with hydrocarbon products. Since some chemical and microbial characteristics of soil were affected, it was observed that some microbial agents such a bacillus sp, pseudomonas sp etc. can degrade and utilize crude oil exposed to the soil environment. Hence, the use of bacteria to remediate soils contaminated with hydrocarbon compounds could be another alternative for soil bioremediation.

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