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

Onyiba, P.O1., Okoh, T2. and Asa, A.A3

1,2,3National Root Crop Research Institute, Umudike Abia State,

Federal Ministry of Agriculture and Rural Development, Nigeria

*princewillogugua@yahoo.com***.**

**ABSTRACT**

This study was carried out to examine the crude oil contamination effect on the rhizosphere soil properties of maize (*Zea mays L*.) and cowpea (*Vigna unguiculata* (L) Walp), grown in a potted sandy loam soil samples. The chemical properties of the soil and microbial dynamics within the rooting region of the crops were determined. The experiment was simply arranged in a completely randomised design (CRD) with four replicates. The factors comprised of four levels of crude oil contamination and two tests crops, with each replication containing eight treatments. The soil was treated with different levels of crude oil; 0% , 0.5%, 2% and 5% v/w. The control samples were not contaminated. Rhizomicrobial population was significantly (p<0.05) diminished with increase in crude oil concentration. At 10 days interval, these species of organisms were isolated which include *Nitrosomonas, Nitrobacter, Rhizobium, Azotobacter, Bacillus, Pseudomonas, Aspergillus, Penicillum, Fusarium, Clostridium, Micrococcus, Enterococcus and Arthrobacter.* However, some group of microbial organisms such as *Bacillus spp, Pseudomonas spp, Micrococcus spp*, *Clostridium spp* persisted after contamination and maintained their potential for degrading petroleum products. As the crude oil contamination increased, it significantly enhanced the population of petroleum degrading bacteria in the two crops which ranges from 2.00 to 9.40 x 108Cfu/g for maize, and 0.50 to 4.90 x 108 Cfu/g for cowpea. At 0.5 to 5% v/w , crude oil contamination of soil diminishes the growth of nitrifying bacteria, nitrogen fixing bacteria, and fungi in soil grown with maize and cowpea crop. The results showed that crude oil contaminations significantly (p<0.05) have negative impact on the pH, mineral elements such as nitrogen, phosphorus and potassium contents, organic content, exchangeable bases and acids, and microbial load of the soils contaminated with petroleum. The observable changes in composition of the mineral elements and microbial load in the soil could be attributed to the toxic nature of the petroleum products. Also, this study has demonstrated that soil contamination with crude oil has a highly significant effect on mineral composition of soils, and can create imbalance in microbial population of the soil leading to infertility of the soil.

**Key words:** Crude oil; Rhizosphere; Soil Properties; *Zea Mays L*.; *Vigna Unguiculata* (L.) Walp.; Microorganisms

**I. INTRODUCTION**

Crude oil contamination and its associated products remain a threat to the soil environment, and as a results a challenging issue in Nigeria and the world at large as crude oil refining activities improve steadily. This challenging issue manifests particularly in the decrease/loss of fertility of agricultural lands, depletion of ecosystems, biodiversity and death of plants, including crops, in the oil producing areas of Nigeria. Crude oil products hinder and retard crops production in crude oil affected areas for varying periods of time, as well as affecting the soil status. According to Chi Yuan *et al* (1995), 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 an increase 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 (Gramss *et al*,1998; 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 sever (Salminen *et al*, 1997).

Nicolotti *et al* (1998)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 (Chin-A-woeing *et al*, 1998).

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 (Nichols *et al*, 1997). 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 (Burken *et al*, 1996).

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 crop (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 (Singh *et al*, 1997; Tarawali *et al*,1997). 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 materials 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.

**II. MATERIALS AND METHODS**

**A. Experimental Location**

The experiment was conducted in the Screen house of Department of crop/soil science, Faculty of Agriculture, Rivers State University, Port Harcourt, Nigeria**.**

**B. Experimental Design**

The study was a 4 x 2 factorial experiment arranged in a completely randomised design(CRD) with four replicates. 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.

C. **Sources of Crude Oil**

The crude oil was 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.

**D. Planting Materials/ Seed Viability**

The viable seeds of *Zea mays* L. and *Vigna unguiculata* (L) Walp, 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( 25-30oC) for 24hrs.Viability tests of the seeds were checked through submerging the seeds in water (by floatation method).

**E. Soil Preparation and Treatment.**

The soil samples 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 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 not contaminated and 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.

**F. Data Collection**

Data collected were soil PH (Electrode method)**,** Organic carbon (Black, 2000), Available phosphorus (Bray *et al*, 1985), 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).

**G. Determination of Microbial Components.**

Microbial components such asNitrifying bacteria, Heterotrophic bacteria, Nitrogen fixing bacteria Hydrocarbon-degrading bacteria, and Fungi which were determined with standard and acceptable agar procedure (Alexander *et al*,1965; Wistreich ,1997).Microbial population countwas also determined as follows;

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

**H. Statistical Analysis**

Research data obtained were simply subjected to analysis of variance (ANOVA), while the

significant means were separated with least significant difference (LSD) at 5% confidence level (p<0.05).

**III. RESULTS**

**A. Effect of Crude oil on soil Physicochemical properties.**

The chemical properties of the rhizosphere soil on the experimental pot before and after contamination are presented in Table.1. The soil, an acidic sandy loamy soil with no previous hydrocarbon content, had originally pH of 6.17, nitrogen 0.134% , avail. phosphorus 69.93 mg/kg and org. carbon 1.84% . On simulation with crude oil, the soil pH was not significantly affected, and organic carbon content of the rhizosphere soil increased significantly with increased level of contamination while available phosphorus significantly reduced to 51.12 and 49.33mg/kg in the 5% soil treatment in maize and cowpea respectively. Also the total nitrogen content was slightly increased in 5% polluted soil to 0.17 and 0.19% for maize and cowpea respectively, 4 weeks after planting.

The pH of the soil before contamination was 6.17 slightly lower than the pH of the soils at the end of the research work for the different treatments. In the 0.5% to 5% rhizosphere treated soils, the pH was slightly increased to 6.26 for maize and 6.26 for cowpea compared to the pH before contamination.The increased levels of crude oil contamination also increased the percentage organic carbon content of the rhizosphere soils of maize to 4.70, and of cowpea to 4.20 compared to the soils before contamination which was 1.84. There was a decrease in available phosphorus with the application of increased crude oil compared to the result of the control for the rhizosphere soil of the crops. The effect of different 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 with the application of crude oil to 51.12 in maize and 49.33 in cowpea. There were significant reductions on the sodium(Na), potassium(K) and magnesium elements in the planted soil of each crops compared to the uncontaminated soil.

The details of the heavy metal concentration in both the contaminated and uncontaminated rhizosphere soil of the crops (maize and cowpea) 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 concentration of lead and cadmium were observed in contaminated soil of maize(5.07 and 1.61) while low concentration was observed in polluted soil of cowpea(4.98 and 0.56). Higher concentration of iron, Fe was observed in polluted maize(1262 ) as compared to unpolluted soil(923.5). Similar trend was observed in cowpea with concentration of 1314. Higher concentration of manganese was recorded in contaminated soil of maize(389.7) while the lowest concentration of manganese was observed in uncontaminated soil of the crops(253.60). Similarly highest concentration of manganese(Mn) was recorded in contaminated soil of cowpea(361.6). Highest concentration of zinc(Zn) was observed in contaminated soil of maize and cowpea(85.76 and 87.6) whereas the lower concentration was observed in uncontaminated soil of the crops(67.55). Higher concentration of copper(Cu) was observed in contaminated soil of maize and cowpea(24.3 and 20.86).

B. **Effect of Crude Oil on the microbial Diversity of the rhizosphere soil.**

Microorganisms of the rhizosphere soil of the crops in oil contaminated and uncontaminated soil were presented in the Table 2. Table 3 also showed the microorganisms isolated from crude oil contaminated and uncontaminated soil whereas Table 4 showed the sensitivity of crude oil degraders in the soil contaminated with crude oil. Crude oil had a significant effect(p<0.05) on the microbial population of the rhizosphere soil of the two crops. There was a significant (p<0.05) decrease in microbial count in the rhizosphere soil of the maize and cowpea with 5 % levels of contamination . For instance, the nitrifying bacteria and nitrogen fixing bacteria counts in the rhizosphere soil of the two crops were significantly reduced to 3.53 × 108 cfu/g and 1.50 x108 cfu/g for maize, and to 1.50x108 and 0.45 x108 cfu/g for cowpea compared to the control of the respective crops. The population densities of the nitrogen fixing bacteria ranged from 5.75 to 0.30× 108 cfu/g while the densities of the organism in maize were from 5.75 to 1.5× 108 cfu/g . The same trend happened in cowpea which was from 3.25 x 108 cfu/g to 0.45 x 108 cfu/g. The rhizosphere of maize and cowpea exposed to 0% - 0.5% (v/w) contamination level recorded the least observable population density of hydrocarbon degrading bacteria whereas 5% contamination level showed the highest result. The results also showed that the initial bacterial densities of 2.00 × 108 cfu/g and 0.50 × 108cfu/g recorded for maize and cowpea in uncontaminated soil when exposed to crude oil contamination increase with time to 9.40× 108 , 4.90 × 108 cfu/g, and 7.0 × 108 over time. The hydrocarbon bacterial counts obtained for legume was 0.50× 108 . 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 unpolluted soil had lower densities of crude oil degraders. Mean density of 0.50 to 4.90 × 108  was recorded for cowpea. Inversely, it was also observed that the microbial population of total heterotrophic bacteria (THB) were lower in crude oil contaminated rhizosphere soil of the test crops. The total heterotrophic bacteria was 1.64 × 108 cfu/g and 1.21 × 108 cfu/g lower in the contaminated soil than in the uncontaminated rhizosphere soil . Fungi count in the rhizosphere of cereal and legume crops in soil contaminated with crude oil is presented in Table 2. The rhizosphere of cowpea was observed to enhance greater fungal growth (2.50 × 107 cfu/g ) compared to that of maize (2.00 × 107 cfu/g) at 5% crude oil pollution. There was however, a significant (p<0.05) decrease of fungal growth on the rhizosphere of the test crops caused by the crude oil contamination of the soil.

**IV. DISCUSIONS**

A significant difference (P≤0.05) was observed between the effects of the crude oil on the rhizomicrobial flora and uncontaminated soil flora (control) of the two test crops studied. A microbial population in the rhizosphere of the cereal and legume in the simulated crude oil contaminated soil was observed as it is seen in Table 2. The crude oil greatly affected soil bacterial and fungal species of these test crops. According to the results of this research work, it was observed that hydrocarbon compounds had a great effect on the total heterotrophic bacteria (THB) counts in the crude oil contaminated soil . It was also observed that there was significant decrease in the 5% level of the crude oil contamination. This occurs as a result of the prevailing unfavourable conditions created by the hydrocarbon compounds which might have reduced the microbial population of THB in the soil. This results confirmed the reports of (Li *et al*, 2007) who also observed that there was a reduction in the heterotrophic bacteria growth in a crude oil-contaminated soil with decrease in the functions of their soil enzymes such as polyphenol oxidase, and dehydrogenase etc. Table 2 showed 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 experiment, most organisms were able to survive the harsh condition at the end of the experiment. Table 5 also shows organisms that utilize/degrade petroleum hydrocarbon and which can survive the toxicity of the crude oil contamination. These microbial agent which can survive high toxicity of crude oil-simulated soil are called Crude oil degraders These organisms that survive were able to use hydrocarbon compounds as a source of energy and food for their survival, growth and multiplication. They have the capacity to degrade the crude oil contaminants and revitalise the environment. Based on the results, the microbial community of the crude oil degraders in the test crops rhizosphere increased overtime, even in soils with the high level (5%) of contamination. However, the uncontaminated soil(control) had lower microbial population of crude oil degraders. Mean population of 2.00 to 9.40 x 108 and 0.50 to 4.90 × 108 were observed and then recorded for maize and cowpea respectively. This occurs due to the presence of hydrocarbon compounds which serves as source of energy for the hydrocarbon degrading bacteria (HDB).Hence, with this it favoured the rapid multiplication of HDB thereby resulting in high population of the organisms in crude oil-simulated soil. The lower microbial population of HDB observed in uncontaminated rhizospere(control) soil was as a result of non-availability of hydrocarbon compounds in the soil which serve as food for them. Moreover, it was observed that gradually HDB increase in some of the treatments in respect to the test crops. This increase happened as a result of the use of the crude oil by crude oil bio-degraders as their energy and carbon source. A report by Roscoe *et al*(1989) noted that there was an increase in the microbial community in a crude oil contaminated soil. Similar findings by Brown,(1995) reported that plant rhizosphere are highly favourable for the proliferation, multiplication and metabolism of microbial because of plant chemical exudates that are released to the soil, hence the increase in rhizobacteria population observed corresponds with increase in amount of nutrient accumulation in the soil. In addition, a gradual increase in soil minerals and nutrients has simply improved microbial growth 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 (Okpowasili *et al*, 1995).

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. However, higher population of nitrogen-fixing bacteria were found in the rhizosphere soil of legume cultured in uncontaminated soil. It was observed that, the increase in the levels of contamination resulted in decrease in the number of nitrogen fixers and nitrifiers with time. Expectedly, nitrogen-fixing bacteria were found in the rhizosphere of the legume(cowpea). Legume (cowpea) exposed to the high (5%) level of contamination harboured the least population of nitrogen fixers. The uncontaminated soil supported the highest counts of 5.65 and 3.25 x 108 cfu/g in maize and cowpea respectively. It was also observed that crude oil generally affected the replication, survival, growth and multiplicity of the nitrogen fixers in the test crops. Moreover, nitrifying bacteria were observed to be sensitive to the crude oil levels that even 2% contamination was significantly different compared to the control. It was reduced with increased concentration of crude oil in the rhizosphere soil of the test crops. It was observed that under the influence of crude oil, nitrifying bacteria could not effectively compete with other microorganisms that grow and multiplied rapidly, leading to the decrease in the available inorganic nitrogen. Similar report and findings confirmed with that of Odu (1981) who noted that aerobic nitrogen fixers relatively multiply abundantly than other microorganisms while nitrifying organisms considerably reduced in population. According to research findings of Muratova *et al* (2003), it was reported that soil simulated with bitumen compounds reduced population count of denitrifying, amonifying, nitrifying, nitrogen fixing bacteria in the rhizosphere soil of plants such as reed and alfalfa.

The results in the table 4 showed that fungal population was observed to reduce with increase in the concentration of crude oil. However, it was observed that in both 0.5, 2% and 5% contamination, the population count of fungi in maize rhizosphere was lower than those in cowpea. Though both rhizosphere soil the crops received the same crude oil treatment, the chemical exudates from the cowpea root must have ameliorated the effect of the crude oil, thus increase microbial count of fungi in its rhizosphere compared to those of the maize. Ekpo *et al* (2007), who had worked on the rhizobacterial and rhizofungal community structure with the use of 165 rDNA fingerprints, noticed that the microbial population 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.

It was also observed in this research work that some bacterial species such as *Bacillus pumilus, Pseudomonas mallci, Enterococcus feacalis, Micrococcus luteus* and all fungal species persisted after the crude oil contamination. 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. The above observation was also confirmed by the report of Yong *et al*, (2006),stating that in a crude oil simulated soil, some bacteria and most fungi have inherent enhanced physiological tolerance and the ability to utilise the crude oil. Some microorganisms, for example *Clostridium* *botulinum, Listeria monocytogen and Rhizobium leguminosarium* were cultured and isolated only before contamination. According to Avidano *et al* (2005), these organisms could have been eliminated because they were able to make use of hydrocarbons as their sole source of carbon and energy.

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 oil. Though Clostridium pasteurianum, Bacillus polymyxa and Pseudomonas aeruginosa maintained their hydrocarbon degrading potentials under prolong contamination to crude oil in soil, most of the nitrogen fixing bacteria including strong biodegraders such as Azotobacter species, together with Nitrosomonas and Nitrobacter with moderate hydrocarbon degrading potential seemed to have lost their degrees of degradability. For further confirmation, the report of 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 population due to the introductions of petroleum waste sludge. Hydrocarbon utilizers possess the ability to tolerate oil contaminated environments because they have the capacity to utilize oil as source of energy( Katsivela *et al*, (2005). In addition, similar reports by Macura *et al*,(1976) confirmed that petroleum waste sludge harmfully affected the microbial community by reducing essential inorganic nutrients and growth factors, and lowering the pH immediately around negatively charged soil surfaces. Obviously, It is observed that only certain nitrogen fixing bacteria have the capacity to entirely grow on nitrogen free media or very low in nitrogen sources (Chibuike *et al*, 2013). The reports backed the result observed in this research work that Bacillus polymyxa, Azotobacter sp, Clostridium pasteurianum and Pseudomonas aeruginosa can grow heavily on crude oil as the sole source of carbon and energy.

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 reduce the soil quality and fertility. It would be observed that the crude oil sealed up soil pore spaces, and as a result preventing the infiltration and movement of water and air into the soil, reducing the biological activities and disrupting biochemical conversions in the soil 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 (Na), iron(Fe) were observed in crude oil contaminated soils Obire *et al* (2002). According to observations made by Okoro *et al*, (2005), soil sodium and iron increased in crude oil-contaminated soils. Crude oil had a dose-dependent inhibitory effect on both the macro and micro elements levels in the soil, with the exclusion of organic carbon which increased as the crude oil was progressively introduced and subsequently increased. There were significant reductions (P<0.05) in the levels of soil Na, K, Mg and Ca with the introduction of crude oil levels, when compared to their levels in the uncontaminated control. Soils contaminated with crude oil is richly endowed with heavy elements such as Fe, Cu, Zn ,Mn, Cd ,Pb and so on (Table 4). A look at the Table 4, high concentration of heavy metals was observed in all contaminated rhizosphere soils. Among all the heavy metals detected in the soil, high concentration of lead (pb) was observed in polluted soil of maize (5.07) while the low concentration of lead was observed in that of Cowpea (4.89) compared to the control. This is a strong prove that maize is a good hyper-extractor which can be used in the process of phytoremediation of crude oil contaminated soil. These heavy elements are characterized by the possession of partially filled d or f orbitals in any common oxidation state (en. Wikipedia.org/wiki/transition-metal). Based on the report of Cunningham *et al,* (1996), through these free orbitals contained in heavy elements, they form coordinate bonds and complexes with ligands such as NH3, H2O, NO2 etc or other elements such as K, Ca, Mg etc (Osuji *et al*, 2007). 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. According to kayode *et al,(*2009), it also reported that cru de oil contaminations decreased the levels of soil nitrate and phosphorus but the effects on other macro-elements remained uninvestigated. 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 (N), phosphorus (P) and potassium(K) but in their work the soil was amended with inorganic fertilizer (N.P.K compound fertilizer).Some of these unessential mineral elements such as Ca, Mg and P are required for seed germination and plant growth. However, this ability of maize to withstand the toxicity of hydrocarbon compounds and grow in concentrations that caused death in 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(es), it must possess the ability and capacity to germinate and grow in the hydrocarbon contaminated soil(s). According to Kayode *et al*, (2009), phyto-remediation could be defined as the process by which biological techniques in terms of the use of plants and their associated micro-organisms are used for the purpose to remediate contaminated soil and water.

**V. CONCLUSIONS**

Microbial populations 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 some microbial growth in sandy loam soil containing the Leguminous plant such as cowpea but high concentrations of oil leads to growth reduction/inhibition in maize and cowpea. It was also observed that high concentrations of oil favour the growth and the survival of crude oil degraders such as Bacillus sp, Pseudomonas sp, clostridium sp, Arthrobacter sp etc. The results gotten from soil chemical analysis imply that crude oil creates adverse and unfavourable condition to the soil health status and composition which made oil spilled soils unsuitable for cropping. The results also showed that crude oil contaminations have unsuitable impacts on the pH, mineral elements (Nitrogen, phosphorus and potassium contents), organic content, and microbial load of soils contaminated with petroleum.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 crude oil exposed to the environment, thereby using bacteria to remediate soils contaminated with hydrocarbon compounds is another alternative.

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

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

Maize, Cowpea ;

LSD0.05 for

pH = ns, ns;

Organic Organic = 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;

**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

**108Cfu/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

**107Cfu/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*  *Nitrobacter vulgaris* | *Azotobacter nigricns*  *Rhizobium phaseoli*  *Rhizobium leguminosarium*  *Bacillus Polymyxa*  *Pseudomonas aeruginosa* | *Aspergillus flavus*  *Aspergillus niger*  *Aspergillus fumigatus*  *Aspergillus aclad*  *Penicillum citrinum*  *Pencillum trequentum*  *Fusarium roseums*  *Tricoderm horizonum*  *Cephalosporium sp* | *Clostridium Pasteurianum*  *C. botilinum*  *Bacillus Polymyxa*  *B. circus*  *B. megatorium*  *B.pumilus*    *Pseudomonas aeruginosa*  *P. avriginosa*  *Micrococcus luteus.*  *Arthrobacter*  *Acinetobacter*  *Flavobacterium* | *Bacillus pumilus*  *pseudomonas mallci*  *Enterococcus feacalis* |

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

|  |  |  |
| --- | --- | --- |
| Isolate Codes | Growth in Crude Oil | Bacterial Isolates |
| A1  B1  B2  A2  A3  C1  D1  C2  D2  D3 | ++  ++  ++  +++  +  +  ++  ++  ++  ++ | *Flavobacterium sp*  *Micrococcuss spp*  *Bacillus Ceresus*  *Pseudomonas aeruginosa*  *Arthrobacter spp*  *Arthrobacter*  *Acinetobacter Spp*  *Clostridium pasteurianum*  *Bacillus polymyxa*  *Azotobacter sp.* |

NB:

+++ = Heavy Growth ;

++ = Moderate growth ;

+ = little Growth.

**REFERENCE**

Agoda, S., Atanda , S., Usange, O.E, Ikotun, I. and Isong, I.U. Crops in soils polluted with crude petroleum hydrocarbons. *African Journal of Agricultural Research*, vol.6 2001,pp.4833-4839.

Alexander M., and Clark F. E. Nitrifying Bacteria. In: Black C.A., Evans D.D., White J.L., Ensminger L.E., Clark F.E. (eds): Methods of Soil Analysis. Part 2. *American Society of Agronomy,* Madison, 1965, pp.1477–1483.

Avidano, L., Gamalero, E., Cossa, G. P. and Carraro, E. Characterization of Soil health in an Italian Polluted site by using micro-organisms as bioindicators. *Applied Soil Ecology*. vol. 30, 2005:pp.21 – 33

Bamidele, J.F. Threats to sustainable utilization of coastal wetlands in Nigeria. *Journal* *of* *Nigeria* *Environmental* *society*. vol. 5(3), 2010:pp.217-225.

Black C.A. Method of Soil Analysis 11. *American* *Society* *of* *Agronomy*, *Modison*.2000, pp. 573-590.

Bray, R.H and Kurtz, L.T

. Determination of total organic and available forms of phosphorus in soils. *Soil Science* , vol.59, 1985: pp.39 -45

Brown, M. E. Rhizosphere microorganisms opportunist bandits or benefactors. In soil Microbiology: A critical Review*. Walker N(Ed) Halsted press*, wiley, New york. 1995, pp. 21-38.

Burken, J.G., and Schnoor, J.L. Phytoremediation: Plant uptake of atrazine and role of root exudates. *Journal* *of* *Environmental* *Quality*. vol. 28, 1996:549-578.

Chikere, B.O, and Okpokwasili, G.C. Enhancement of biodegradation of petrochemicals by nutrient supplementation. *Nigerian Journal of Microbiology*, vol.17(2), 2003: 130-135.

Chibuike, G.U. and Obiora, S. C. Bioremediation of hydrocarbon-pollution soils for improved crop performance. *International* *Journal* *of* *Environmental* *Sciences*, Vol. 4(3),2013, pp. 223-237.

Chin-A-woeing, T.E.C., Bloemberg, G.V, Van der Bij, A.J., Van der Drift ,K.M, Schripsema, J. Kroon, B., Scheffer, R.J., Thomas, J.E., Luteernberg, B.J. Biocontrol by phenazine-1-Carbonxamide-producing pseudomonas chlororaphis PCL 1391 of tomato rot caused by *fusarium oxysporum* F. sp. *Radicis lycopercisi*. *Molecular* *Plant* *microbe* *interaction*. vol.11, 1998:pp.1069-1077.

Chi Yuan, F., and Krishnamurthy, M. Enzymes for enhancing bioremediation of petroleum contaminated soils: A brief review. *Air* *waste* *management* *Association*. vol.45, 1995: pp.453-460

Cunningham, S.D., Anderson, T.A., Schwab, P.A., and Hsu, F.C. Phytoremediation of soils contaminated with organic pollutants. *Advanced Agronomy*, vol. 56, 1996: pp.55-114.

Ekpo, M.A., and Thomas, N.N. An investigation on the state of microorganisms and fluted pumpkin(*Telfairia occidentalis*) in a crude oil impacted garden soil. Nigerian Journal of Microbiology. vol.21, 2007: pp.1572-1577

Eze, C.N., Maduka, J.N., Ogbonna, J.C and Eze, E.A.Effects of Bonny light crude oil contamination, shoot growth and rhizobacterial flora of *Vigna unguiculata* and *Arachis hypogea* grown in sandy loam soil. *Science Research* .*Essential*. vol. 8(2), 2013. pp.99-107

Gramss, G., Gunther, T.H., and Fritsche, W., Spot tests for oxidative enzymes in ectomycorrhizal, wood- and litter decaying fungi, *mycological* *research*,vol.102, 1998, pp. 67-72.

Hofman, J., Svihalek, J., and Holoubek, I. Evaluation of functional diversity of soil microbial communities- a case study. *Plant,* *Soil* *and* *environment*, vol. 50, 2004, pp. 141-148.

Katsivela, E., Moore, E. R. B., Maroukli, D., Strompl, C., Pieper, D. and Kalogerakis, N. Bacterial community dynamics during in-Situ bioremediation of petroleum waste sludge in land farming sites. *Biodegradation*. vol. 16, 2005:pp. 160-180.

Kayode, J., Oyedeji, A. A. & Olowoyo, O. Evaluation of the effect of pollution with spent lubricating oil on the physical and chemical properties of soil. *The Pacific Journal of Science and Technology. vol.*1(10), 2009:pp. 387-391.

Langyinto, A.S, Loweberg-Deboer, J., Faye, M., Lambert, D.,Ibro, G., Moussa,B., Kergna, A., Kushwaha, S.,Musa, S. and Ntoukam, G. (2003) Cowpea supply and demand in west and central *Africa*. *Field* *Crop* *Research*,vol.82, 2003:pp. 215-231.

Li, H.Y, Zhang, I. , Kravchenko, H. Xu, and Zhano, C. Dynamic changes in microbial activities and community structure during biodegradation of petroleum compound: A laboratory experiment. *Journal of Environmental Science., vol.* 19, 2007:pp.1003-1013.

Lin, Q., and Mendelsshohn, L.A.(2009) Potential of restoration and phytoremediation with *juncus roemerianus* for diesel-contaminated coastal wetlands. *Ecological Engineering*. vol. 35,2009:pp.85-91.

Macura , L. and Kunes , F. Foliar microbiology. *Praune*; vol.6,1976:pp.398–407.

Muratova, A., Naruta, N., Wand, H.,Turkovskaya, O., Kuschlc, P., John R, and Merbach, W. Rhizosphere microflora of plants used for the phytoremediation of bitument-contamination. *Microsbiological Research* vol.158,2003:pp.151-161.

Milic, J.S., Beskoski, P.V., Milic, V.M., Ali, S.A.M., Goigic-Cvijouic, G.D., and Vrvic, M.M. Bioremediation of soil heavily contaminated with crude oil and its products: composition of the microbial consortium. *Journal of the Serbian Chemical Society*. vol.74(4), (2009), pp. 455 - 460.

Nichols, D., Wolf, D.C., Rogers, H.B., Beyrouty, C.A., and Reynolds, C.M. Rhizosphere microbial populations in contaminated soils. *Water Air Soil Population*.vol.95,1997:pp.165-178.

Nicolotti, G., and Egli, S. Soil Contamination by Crude oil: Impact on the mycorrhizosphere and on the vegetation potential of forest trees, *Environmental* *pollution*,vol.99,1998. pp.37-43.

Obire, O., and Nwaubeta, O. Effects of refined petroleum hydrocarbon on soil physicochemical and bacteriological characteristics, *Journal* *of* *applied* *science* and *environmental* *management*, vol.6(1),2002, pp. 39-44.

Odu, C. T. I. Microbiology of soil contaminated with petroleum hydrocarbon. In Extent of contamination and some soil and microbial properties after contamination. Journal of institute Pollution. vol.7,1981:pp.279-286.

Okoro, J.C., Amadi, E.N., and Odu, C.T.I (2005). Effects of soil treatments containing poultry manure on crude oil degradation in sandy loam soil. *Journal of Applied Ecology and Environmental Research*, vol.3(1), 2005: pp.47-53.

Okpokwasili, G.C, and James, W.A. Microbial contamination of kerosene, gasoline and crude oil and their spoilage potentials. *Mat. Org*., vol. 29, 1995: pp.147- 156.

Osuji, L. C. & Nwonye, I. An appraisal of the impact of petroleum hydrocarbons on soil fertility: The Owaza experience. *African Journal of Agriculture,vol.* 2(7), 2007:pp.318-324.

Oyewo, I.O. Technical Efficiency of maize Production in Oyo state. *Journal* *of* *Economics* *and* *international* *finance*, vol.3,2011, pp.211-216.

Robertson, S. J., McGill, W.B., Massicotte, H. B., and Rutherford, P. M.,.Petroleum hydrocarbon contamination in boreal forest soils: A mycorrhizal ecosystems perspective, *Biological* *reviews*, vol.82, 2007, pp.213-240.

Roscoe, Y.L, Mcgill, W.E, Nbory, M.P, Toogood, J.A. Method of accelerating oil degradation in soil. In: Proceeding in workshop on reclamation of disturbed Northern Forest, research Center, Alberta, Edmonton, 1989, pp. 462-470.

Salminen, J., and Haimi, J.,(1997).Effects of pentachlorophenol on soil organisms and decomposition in forest soil, *Journal* *of* *applied* *ecology*, vol.34,1997. pp.101-110.

Seglers, D., Bulcke, R., Reheul, D., Siciliano, S. D., Top, E.M and Verstraete, W. Pollution induced community tolerance (PICT) and analysis of 16S RNA genes to evaluate the Long-term effects of herbicides on methanotrophic communities in soil, *European* *journal* *of* *soil* *science*,vol.54, 2003. pp.679-684.

Singh, B.B., Ehlers, J.D., Sharma, B., and Freire Filho, F.R. Recent progress in cowpea breeding. In: Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. and Tamo, M.Eds., *Proceedings, the world Cowpea Conference III, Challenges and opportunities for enhancing sustainable cowpea production*, International Institute of Tropical Agriculture (IITA),Ibadan,2002, 22-40

Singh, B.B. and Tarawali, S.A.Cowpea and its improvement: key to sustainable mixed crop/Livestock farming systems in west Africa. In: Renard, C. Ed, Crop Residues in sustainable mixed crop/Livestock farming systems, *CAB* *international*, 1997, pp.322

Tarawali, S.A., Singh, B.B., Peter, M., and Blade, S.F.

Cowpea haulms as fodder. In: Singh, B.B., Mohan Raj, D.R., Dashwall, K.E. and Jackai, L.E.N (Eds).Advances in Cowpea Research. International Institute of Tropical Agricultural (IITA) and Japan International Research center for Agricultural Sciences (JIRCAS),IITA,Ibadan,1997, pp.375.

United states Agency for international Developmental(USAID).Package of practices for maize production. Washington DC,2010,pp.1-23.

Valenzuela, H. and Smith, J.. Cowpea: Cooperative extension service. College of Tropical Agriculture and Human Resources, University of Hawai at Manoa, Honolulu,2002, pp.1-4.