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

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**ABSTRACT**

The research trial was carried out to assess the crude oil contamination effect on the rhizosphere soil properties of maize (*Zea mays L*.) and cowpea (*Vigna unguiculata* (L) Walp), planted in a sandy loam soil. The soil chemical properties and microbial dynamics within the rooting zone of the crops were significantly determined. The trial was simply arranged in a completely randomised design (CRD) with four replicates. The factors consisted four levels of crude oil contamination, 0% , 0.5%, 2% and 5% v/w, and two tests crops,*Zea Mays* and *Vigna Unguiculata* ,with each replication containing eight treatments. The soil sample was treated with different levels of crude oil contamination, while the control remained uncontaminated. The results showed that rhizomicrobial load was significantly (p<0.05) diminished as crude oil concentration in the soil sample increases. At 10 days interval, these species of organisms were isolated; *Nitrosomonas, Nitrobacter, Rhizobium, Azotobacter, Bacillus, Pseudomonas, Aspergillus, Penicillum, Fusarium, Clostridium, Micrococcus, Enterococcus and Arthrobacter.* Further, few microbial organisms such as *Bacillus spp, Pseudomonas spp, Micrococcus spp*, *Clostridium spp* persisted in the soil after crude oil contamination had been introduced, thereby indicating that they possess the capacity to degrade crude oil products. As the crude oil contamination increased, population of crude oil degrading bacteria increased as well, ranging from 2.00 to 9.40 x 108Cfu/g in the soil of maize crops, while 0.50 to 4.90 x 108 Cfu/g in the soil of cowpea crops. At 0.5 to 5% v/w, crude oil contamination of soil significantly reduced the bacteria counts of nitrifying bacteria and nitrogen-fixing bacteria, and fungi counts in the soil grown with maize and cowpea crops respectively. Also, the results showed that crude oil contaminations significantly (p<0.05) have adverse impact on the soil pH, mineral nutrients of nitrogen, phosphorus and potassium, organic carbon contents, exchangeable bases and acids, and soil microbial load contaminated with crude oil products. Changes in mineral nutrient contents and microbial load in the soil could be attributed to the toxic nature of the crude oil products. Therefore, this trial had demonstrated that soil contamination with crude oil had a high significant effect on the mineral nutrients of the soils as well as changes in soil microbial population leading to lost of soil fertility.

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

**I. INTRODUCTION**

Crude oil contamination and its associated products pose a big challenge to the agricultural soils in the oil producing areas of Nigeria and the world as crude oil refineries improve steadily. This challenging issue is evident, particularly to the loss of soil fertility of agricultural lands, decline in ecosystem functions and services, depletion of biodiversity and death of crop plants. Crude oil products negatively affect crop productions as well as the soil health status for a varying time. Chi Yuan *et al*(1995) revealed that crude oil hydrocarbons consist aliphatic, oleic, naphthenic and polycyclic aromatic hydrocarbons, which could transform soil physical, chemical, biological including ecological characteristics of the soil. These hydrocarbon compounds are adversely harmful, obnoxious and toxic in nature, and are greatly responsible for changed agricultural soil fertility. The contaminated soils lose their resilience, and could require time to recover their fertility (Wyszkowska *et al*, 2001). Eze *et al*, (2013) noted that one of the ways contaminated soils loss their biological activities is via inhibition of microbial activities. Contaminated Soils differ from the uncontaminated virgin soils as a result of their soil physicochemical as well as their biological properties (Robertson *et al* , 2007). A hydrocarbon contaminated soils occurred due to a change in organic carbon content, and biological activities which could lead to an initial reduction in soil biodiversity when compared to soils that have not been previously contaminated.

Hofman *et al*(2004) observed that there are increase in soil microorganisms in crude oil polluted soils, and that with time different species of organisms often decrease or increase. As microbial organisms are being reduced, others are rapidly evolving showing their capacity to degrade the soil contaminants (Gramss *et al*,1998; Seglers *et al*,2003). The introduction of crude hydrocarbon compounds interfere with plant-fungus mutual relationships as it alters the soil environment preventing the transportation of diffusible chemical signals (auxins). Salminen *et al*, (1997), noted that the toxicity of hydrocarbon compounds in the humus soils with microorganisms have been found to be less sever. Some soil microbial agents may tolerate, and resist crude oil hydrocarbons thereby degrade these compounds to benefit them and plants. Soil microorganisms especially fungi develop the ability to degrade these contaminants which they utilise as the source of energy and food for their survival and growth(Nicolotti *et al* ,1998). Of a particular interest, these microbial agents; rhizo-bacterial flora such as nitrifying bacteria and nitrogen fixing bacteria, petroleum degrading bacteria and fungi have numerous beneficial roles in the soil environment. They have the ability to assimilate and degrade crude oil pollutants, and also act as bio-fertilizers(by fixing nitrogen), phyto-stimulation and bio-control of soil-borne plant diseases (Chin-A-woeing *et al*, 1998).

Most of these organisms inhabit the root zones of plants. The region of soil around the plant roots is simply called rhizosphere. The rhizosphere harbours greater diversity of microorganisms compared to soils with no plant (Nichols *et al*, 1997). Plant roots exude chemical compounds like enzymes, aliphatic, aromatics, amino acids, sugars and low molecular weight carbohydrates that attract the proliferation of microbial activities by giving nutrients to the organisms (Burken *et al*, 1996).

Crude oil hydrocarbon products are injurious to the soil properties and adversely affect crop production (Bamidele, 2010;Lin *et al*, 2009). Obviously, the two test crops used here are maize *(Zea mays*) and cowpea (*Vigna unguiculata*). They are staple crops used significantly for food, and livestock feeds during the dry seasons in different parts of Nigeria (Singh *et al*, 1997; Tarawali *et al*,1997). Among the crops, cowpea is a leguminous crop which 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. Cowpea (*Vigna unguiculata*) 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).The crop is also used as a cover crop to curtail weeds in agricultural lands

(Valenzuela *et al*, 2002). Maize (*Zea mays*) is one of the major staple cereal crops planted for food, feed and for industrial purposes (USAID, 2010; Oyewo, 2011). Maize contains carbohydrate and vitamins in human food. Vitamins like C and A can be sourced from white and yellow grains respectively. Maize grain can also be used as livestock feeds, and also serve as a raw materials for starch, flour and alcohol production (Agoda *et al*, 2011). Therefore, the research trial was to evaluate the effect of crude oil contaminations on the rhizosphere Soil of *Zea Mays L*. and *Vigna Unguiculata* (L.), with respect to the soil chemical attributes and microbial dynamics within the rooting region of planted soil.

**II. MATERIALS AND METHODS**

**A. Experimental location**

The experiment was conducted in 2016 planting season at the screen house of department of crop/soil science, Faculty of Agriculture, Rivers State University, Port Harcourt, Nigeria**.**

**B. Experimental design**

The research was arranged in a completely randomised design with four replicates. The factors consisted four levels of crude oil contamination, 0%, 0.5%, 2% and 5% and two test crops, maize and cowpea, with each replication containing eight perforated experimental pots.

C. **Crude oil and Soil Sample sources**

The crude oil hydrocarbons were sourced fresh from 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 sample had no previous exposure to crude oil contamination.

**D. Planting Materials.**

The viable seeds of *Zea mays* L. and *Vigna unguiculata* (L) were obtained from International Institute of Tropical Agriculture(IITA), Ibadan and Rivers State Agricultural Development Programme (ADP) respectively. They were stored at a room temperature, 25-30oC for a day. The seed viability tests were checked via submerging the seeds in water i.e. by floatation method.

**E. Soil Preparation and Treatment.**

The soil samples were collected, air-dried, sieved and dispensed in 5kg weights into thirty-two (32) plastic perforated buckets at the bases. The perforated buckets were divided into four replications of eight each, and each replication was used for the two text crop plants. Each perforated bucket, with the soil samples, was contaminated with one of four different levels of light crude oil, 0% , 0.5% , 2.0% , and 5% v/w. All the control samples were not contaminated and planted. All the buckets, with soil samples and crude oil, were kept 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 by Electrode method**,** organic carbon (Black, 2000), available phosphorus (Bray *et al*, 1985), total nitrogen by modified kjeldahl method, heavy metal content by atomic absorption spectrophotometer, exchangeable bases such Calcium, Manganese, sodium, potassium by titration method and flame photometer and exchangeable acids by titrimetric method.

**G. Determination of Microbial Components.**

Microbial components such asnitrifying bacteria, heterotrophic bacteria, nitrogen fixing bacteria, hydrocarbon-degrading bacteria, and fungi 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 analyzed and 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. Crude oil contamination and rhizospheric soil physicochemical properties.**

The rhizospheric soil physicochemical properties from the experimental pot before and after contaminations are presented in Table.1. The soil, an acidic sandy loamy soil with no previous hydrocarbon content, had originally 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 with increased level of contaminations while available phosphorus reduced to 51.12 and 49.33mg/kg at 5% soil treatment for maize and cowpea respectively. Also the total nitrogen content was slightly increased at 5% soil treatment to 0.17 and 0.19% for maize and cowpea respectively, 4 weeks after planting.

The soil pH, before contamination, 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 with the application of crude oil 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.

B. **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 × 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 control of the respective crops. The population densities of the nitrogen fixing bacteria were ranged from 5.75 to 0.30× 108 cfu/g while the densities of the organism in the soil of maize were from 5.75 to 1.5× 108 cfu/g . The same trend happened in the soil of cowpea which were from 3.25 x 108 cfu/g to 0.45 x 108 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 × 108 cfu/g and 0.50 × 108cfu/g recorded for maize and cowpea in uncontaminated soil when exposed to crude oil contamination increased, with time, to 9.40× 108 , 4.90 × 108 cfu/g, and 7.0 × 108 . The hydrocarbon bacterial counts obtained for legume were 0.50× 108 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 × 108 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 × 108 cfu/g and 1.21 × 108 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 × 107 cfu/g compared to that of maize, 2.00 × 107 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.

**IV. DISCUSIONS**

**A. Crude Oil contamination and microbial Diversity of the rhizosphere soil.**

A significant difference (P≤0.05) was observed between the effects of the crude oil on the rhizomicrobial flora and uncontaminated soil flora of the two test crops studied. The microbial populations in the rhizosphere of the cereal and legume 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 were 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 agent 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 108 cfu/g and 0.50 to 4.90 × 108  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. Roscoe *et al*(1989) noted that there was an increase in the multiplications of microbial communities in a crude oil contaminated soils. In the same vein, Brown,(1995) 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, a gradual increase 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 (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.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 108 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. Odu (1981) 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, Macura *et al*,(1976) 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 (Chibuike *et al*, 2013). 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.

**B. Crude oil contaminations and rhizospheric soil physicochemical 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 reduce 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 contains 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 was 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 compounds. Cunningham *et al,* (1996) reported that 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 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, 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.

**V. CONCLUSIONS**

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 sp, 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.

**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.

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