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Research Paper

PHYTOREMEDIATON POTENTIAL OF *VIGNA UNGULCULATA* (L) WALP IN CRUDE OIL POLLUTED SOIL IN ELEME RIVERS STATE, SOUTH-SOUTH NIGERIA

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Abstract

The Evaluation of Growth Performance of Cowpea *(Vigna unguiculata*) (L) *Walp* in crude oil polluted soil was investigated using four (200ml, 400ml, 600ml and 800ml) levels of crude oil including control(no pollution) in Completely Randomized Design of 3 replicates. 0.5% of poultry manure was added to the soil at 7days after pollution and the soil left for 14days after application of manure before planting. Growth parameters such as plant height, number of leaves, leaf area, and petiole length were measured at interval of 3 weeks after planting (3WAP) and physicochemical parameters such as pH, electrical conductivity, nitrogen, potassium, phosphorus and total petroleum hydrocarbon were also analyzed. The results revealed that plant height was significantly affected at ($p \leq 0.05$) especially in 600ml and 800ml polluted soil compared to other treatments. All the treatments reduced the number of leaves, petiole length, leaf length and width significantly. Results showed that crude oil affected the overall growth performance of cowpea. Also the concentration of N, P, and K increased with higher oil concentration. There was a significant decrease in the TPH of soil from 19686mg/kg to 903mg/kg and an increase in the uptake of the contaminant by the plant from 1540mg/kg to 2012mg/kg after 9 weeks of the experiment. From the study, *Vigna unguculata* absorbed more at lower pollution (200ml-600ml) than at 800ml pollution. Thus, the plant showed great promise in the phytoremediation of crude oil contaminated soil.

Key words: Phytoremediation, *Vigna uguculata,* pollution, soil, Total Petroleum Hydrocarbon.

INTRODUCTION

Technological advancement in terms of industrial activities has caused a rapid rise in petroleum consumption and, as a result, a huge amount of hydrocarbons are being discharged into the environment, either deliberately or accidentally every year. There has been many reports of oil spills worldwide. For instance, there was a crude oil spill of 0.04 mega tonnes into Prince William Sound, Alaska in 1989 (Swannell *et al.*, 1996).The Department of Petroleum Resources estimated 1.89million barrels of petroleum were spilled into the Niger Delta between 1976 and 1996 out of a total of 2.4million barrels spilled in 4,835 incidents approximately 220 thousand cubic meters (Vidal, 2010). Soil and water contamination by crude petroleum and refinery products is an ever growing problem in Nigeria as oil mining and refining activities increase steadily (Vidal, 2010). This problem manifests particularly in the loss of fertility of agricultural lands and death of plants, including crops, in the oil producing areas of Nigeria. A report by Adoki and Orugbani (2007) shows that oil pollution prevents crop growth and yield in those areas for varying periods of time. Crude oil negatively affects the germination, shoot growth and yield of most plant species including seashore plants (Bamidele and Igiri, 2011) and field grasses (Debojit *et al.*, 2011). Crude oil and its products are made up of aliphatic, oleic, naphthenic and aromatic hydrocarbons, which change the physical and chemical properties of soil and its structure (Chi Yuan and Krishnamurthy, 1995). These compounds are largely responsible for altered fertility of soil. Atuanya (1987), Agbogidi and Ejemete (2005) noted that oil in soil has deleterious effects on the biological, chemical and physical properties of the soil depending on the dose, type of the oil and other factors. Benka-Coker and Ekundayo (1995) also reported that the microbiological components of soil are usually negatively affected when oil is applied to soil.

One of the environmental challenges posed by oil pollution is the alteration in the physical and chemical nature of the soil which subsequently affects the growth of plants (Chronopoulos *et al*., 1997). Petroleum hydrocarbon contamination may affect plants by retarding seed germination and reducing height, stem density, photosynthetic rate and biomass or resulting in complete mortality (Pezeshki *et al*., 2000). Phytoremediation has emerged as a viable option for the remediation of petroleum hydrocarbon polluted sites (Tanee and Kinako, 2008).

In majority of studies, grasses and legumes such as bush beans (*Phaseolus vulgaris* L.), Switch grass (*Panicum virgatum*), alfalfa (*Medicago sativa* L.) and sorghum (*Sorghum bicolor*) have been known for their potentials to facilitate the phytoremediation of sites contaminated with petroleum hydrocarbons (Reilly *et al*., 1996; Qiu *et al*., 1997). The use of phytoremediation as a cleanup option may not only degrade contaminants but could enhance habitat recovery through the stimulation of vigorous vegetative plant growth (Lee *et al*., 2001).

Cowpea (*Vigna unguiculata* L.) belongs to the family *Fabaceace*. The seeds are edible as it contains proteins. It is mostly grown in crop rotation to replenish lost fertility especially nitrogen in the soil.

Vigna unguiculata is important in faming system because of its ability to fix nitrogen in the soil which is always a limiting factor in crude oil polluted soil (Tanee and Akonye, 2009), hence its use in is a propriety the remediation of crude oil polluted soil as oil spillage is one of the challenges in the agricultural lands of the Niger Delta Regions.

Nigeria, over the years has experienced environmental pollution of different forms (land, water, air) and all efforts have been made by both governmental and non-governmental organizations in the drive towards finding a lasting solution to this menace (Okoh, 2010). The influx of companies (locals and multinationals) engaged in all manner of activities across the length and breadth of the country impact negatively on our environment(Okoh, 2010). Hydrocarbon management is a fundamental environmental management tool especially in mining companies that deal with large volumes of hydrocarbons and hydrocarbon related wastes (Okoh, 2010).Contamination of soil with petroleum hydrocarbons is as a result of oil excavation and shipping and is a potential threat to production agriculture.

Oil contaminated soils is a concern because the contaminated soils are unsuitable for agricultural, industrial, or recreational uses and are potential sources for surface and ground water contamination (Okoh, 2010). This contamination causes a hindrance to agriculture which happens to be the major source of food in Nigeria and all over the world (Okoh, 2010). With the expanding population of the country, shortages of food could lead to poor health and poor standard of living. Recent public concern and rising costs regarding the conventional clean-up procedures show the need for a less expensive bioremediation option such as phytoremediation (Okoh, 2010).

This research work aims at assessing the effiency of phytoremediation technology of crude oil contaminated soil using Cowpea (*Vigna unguiculata*) as an alternative to the conventional chemical process being practiced in Nigeria. To examine the effectiveness of *Vigna unguiculata* in the remediation of crude oil contaminated soil.

OIL POLLUTION

Oil spillage has a major impact on the ecosystem into which it is released and may constitute ecocide (any extensive destruction of the natural environment and disruption of ecosystem to such an extent that the survival of the inhabitants of that territory is endangered (Baird, 2010).
Damages to ecosystems by oil pollution vary according to the types of habitats receiving oil. A number of physiological processes in plants such as transpiration, respiration, photosynthesis and translocation are affected adversely by oil contamination (Baker, 1971). Oil spill on land can result in direct adverse effects to contacted vegetation (Ginsburgh, 1931). Oil contaminated soil may become anaerobic and reducing conditions can result in increased solubilites of iron(Fe) and Manganese(Mn) to the extent that these potentially phytotoxic elements are absorbed by roots/plants (Gidden,1976; Volk, 1980). Oil spill on terrestrial environments can cause sensitive species to become extinct and tolerant species dominate. Overall plant cover can be reduced and some areas may remain barren (Freedman and Hutchinson, 1976; Kinako, 1981).Oil pollution also contributes indirectly to other aspects of habitat deterioration such as sheet erosion (Kinako, 1981). It is generally accepted that lack of vegetation cover, disrupted soil structure and reduced moisture holding capacity of oil polluted soil can all contribute to erosion (Kinako, 1981).

LITERATURE REVIEW

Phytoremediation

Phytoremediation, the stimulation of contaminant degradation by the growth of plants and their associated microorganisms, is emerging as a potentially cost-effective option for the cleanup of petroleum hydrocarbons in terrestrial environments (Banks *et al*., 2000) and is thus defined as the use of plants to extract, sequester, or detoxify pollutants (Frick *et al*., 1999; Banks *et al*., 2000). This remediation method is environmentally friendly and visually attractive, and the structure of the soil is highly maintained (Khan *et al*., 2000).
Phytoremediation uses plants to clean up pollutants in the environment. Plants can help clean up many kinds of pollutants including metals, pesticides, explosives, and oil. The plants also help prevent wind, rain, and groundwater from carrying pollutants away from sites to other areas (Khan *et al*., 2000).
Phytoremediation works best at sites with low to medium amounts of pollution (Khan *et al*., 2000). Plants remove harmful chemicals from the ground when their roots take in water and nutrients from polluted soil, streams, and groundwater. Plants can clean up chemicals as deep as their roots can reach (Khan *et al.*, 2000).
Phytoremediation can occur even if the chemicals are not taken into the plant by the roots. Chemicals can stick or *sorb* to plant roots or they can be changed into less harmful chemicals by bugs or *microbes* that live near plant roots (Khan *et al.*, 2000). The plants are allowed to grow and take in or sorb chemicals (Khan *et al.*, 2000). Afterward, they are harvested and destroyed, or recycled if contaminants (e.g. metals) stored in the plants can be reused (Khan *et al.*, 2000).
Phytoremediation is hypothesized to be particularly effective when used together with nutrient enrichment because hydrocarbon contamination may result in nutrient deficiencies in the contaminated sediment. Added fertilizers could increase the rate of oil degradation by indigenous microorganisms in the rhizosphere and simultaneously stimulate plant biomass production, thereby increasing the effectiveness of phytoremediation (Zhu *et al*., 1999).

Mechanisms of Phytoremediation

Phytoremediation utilizes physical, chemical, and biological processes to remove, degrade, transform, or stabilize contaminants within soil and groundwater. Each of the above mechanisms will have an effect on the volume, mobility, or toxicity of contaminants, as the application of phytoremediation is intended to do (Banks *et al*., 2000).
Phytoextraction The first phytoremediation patent applied for in the United States related to phytoextraction (McCutcheon and Schnoor, 2003). Phytoextraction refers to the ability of plants to remove metals and other contaminants from the subsurface and translocate them to the leaves or other plant tissues. The plants may then need to be harvested and removed from the

site. Even if the harvested plants must be landfilled, the mass disposed of is much smaller than the original mass of contaminated soil. Incineration and disposal of the plants is cheaper than traditional remediation methods. As a comparison, it is estimated that a site containing 5000 tons of contaminated soil will produce only 20-30 tons of ash (Black, 1995). Use of phytoextraction is usually limited to metals and other inorganic compounds in soil or sediment (Black, 1995).

Phytoremediation as a Potential Remediation Technology Numerous benefits of phytoremediation have been established or hypothesized:

Phytoremediation can be less invasive and destructive than other technologies (Ebbs *et al*., 1997). Studies have indicated that implementing phytoremediation may result in a cost savings of 50 to 80 percent over traditional technologies (Ebbs *et al*., 1997). Phytoremediation may provide habitat to animals, promote biodiversity, and help speed the restoration of ecosystems that were previously disrupted by human activity at a site (Wilson, 2004). Phytoremediation installations can improve the aesthetics of brownfields or other contaminated sites. Phytoremediation may promote better air or water quality in the vicinity of the site. Vegetation may help reduce erosion by wind or water (Wilson, 2004). Planted trees may also provide shade to buildings, helping to decrease energy consumption (Nowak and Crane, 2002). The plants can be easily monitored (Nowak and Crane, 2002).

Cowpea (*Vigna unguiculata*) The plants used in this study (cowpea) is a staple leguminous crops in different parts of Nigeria. Legumes play an important role in the restoration and sustenance of soil fertility through their ability to fix atmospheric nitrogen in partnership with certain bacterial species (Anoliefo *et al.*, 2006).

Owing to this overriding role of legumes in agriculture, their growth and survival in arable lands become paramount, particularly now that many farm lands are fast losing their agricultural value due to pollution and overuse (Anoliefo *et al.*, 2006). Cowpea is an annual, herbaceous legume (Anoliefo *et al.*, 2006). It is a short-day crop sensitive to chilling temperatures but adapted to warm weather and humid
conditions (Asumugha, 2002; Islam *et al.*, 2006). It belongs to the family *Fabaceae* and sub-family *Faboidea*; Tribe *Phaseoleae*, Sub-tribe *Phaseolinae* (Padulosi & Ng. 1997). All cultivated cowpeas are grouped under the species *Vigna unguiculata*, which is sub-divided into four cultivar groups: *unguiculata*(the common cowpea), *Biflora* (the catjang), *sesquipedalis* (the yard-long bean) and *textilis* (used for fibers) (Reis & Frederico, 2001; Singh *et al*., 1997).

It originated from central Africa but it is now widely cultivated in many parts of the tropics and subtropics including west Africa and India (Olaleke *et al.*, 2006). The leaves of cowpea are eaten in salad and the immature pods are used as vegetable. The grains are rich source of plant protein to man; they contain mineral salts, vitamins and fats (Ogbo, 2009). The young shoots are eaten like spinach (Adepoju and Marcus*,* 2000) while the immature seeds are eaten fresh, frozen and canned.

It is regarded as poor man's meat because they are the cheapest source of protein; essential amino acids in them are also in sufficient amount (Biradar *et al*., 2007; Awe, 2008; Omotugba *et al*., 2008). The plant has a lot of industrial potentials (Lambot, 2003).

Cowpea and Phytoremediation

Cowpea is a multipurpose crop, providing food for human and feed for livestock, It can also be used as a cover crop (Langyintuo *et al*., 2003; Singh 2002; Timko *et al*., 2008). The very early maturity characteristics of some cowpea varieties provide the first harvest earlier than most other crops during production period. This is an important component in hunger fighting strategy, especially in the Sub-Saharan Africa where the peasant farmers can experience food shortage a few months before the maturity of the new crop. Its drought tolerance, relatively early maturity and nitrogen fixation characteristics fit very well to the tropical soils where moisture and low soil fertility is the major limiting factor in crop production (Hall, 2004; Hall *et al*., 2002). This crop is grown worldwide with an estimated cultivation area of about 12.5 million hectares annually and an annual worldwide production of over 3 million metric tons (Li *et al*., 2001). About 70% of the cowpea production occurs in marginal areas of West Central,

East and Southern Africa. Nigeria is the largest producer and consumer of cowpea at estimated annual yields of 2 million metric tons (Singh *et al*., 2002; Timko *et al*., 2008).
Cowpea was chosen for this study because of its natural ability to fix atmospheric nitrogen. It is an efficient nitrogen fixing, heat and drought-tolerant legume (Bittenbender, 1990), for this reason, they do not have to compete with the indigenous bacteria over the existing nitrogen because they can produce their own nitrogen for growth, thereby, facilitating the process of phytoremediation. Africa is the Origin of Cowpea (Angesse, 2006) with Nigeria being one of the major cowpea growing countries in the world (Singh *et al*., 2000)

MATERIALS AND METHOD
Crude oil
Bonny light crude oil was obtained from Nigerian National Petroleum Corporation (NNPC) Port Harcourt Refinery, Alesa – Eleme, Rivers state, Nigeria. The crude oil was cooled obtained fresh from the production plant and collected with sterile containers.
Viable seeds of the leguminous crop, vegetable cowpea (*Vigna unguiculata)* was purchased at Ihiagwa Market in Owerri, Imo state and stored at room temperature (25 to 30°C) for not more than 24hrs. Soil sample was collected from an agricultural soil in Federal University of Technology, Owerri farmland using sterile containers. The soil was mixed thoroughly and sieved with a 2mm mesh sieve. 2000g of soil was placed in each polythene bags. The bags were perforated at the sides and bases to avoid water logging and also increase soil aeration. The soil was analysed at the beginning and end of the experiment to determine the initial and final levels of hydrocarbon parameters.

Pollution of Soil with Crude Oil
20kg of soil were filled in 75 poly bags each treatment had 5 bags with 3 replicates (5 different treatments with 3 replicates) (5x5x3) =75. The bags were arranged in a Completely Randomized Design consisting of five treatments replicated three times with different concentrations of crude oil (0, 200ml, 400ml, 600ml and 800ml) respectively for each treatment, was poured evenly on the surface of the soil (surface pollution), using sterile measuring cylinder. The crude oil was thoroughly mixed with a trowel to achieve uniformity. The control was not polluted with crude oil. The polluted soils was allowed to stay for one week before planting to enable the volatile compounds present to escape (0.5%) 5kg of poultry manure was added to each bag after 7days of pollution and then allowed to incubate for fourteen (14days) before planting the cowpea seeds. Samples of soils were analyzed before surface pollution and after surface pollution for physiochemical analysis.

Procedure
The land area where the bags were kept was cleared with a cutlass and hoe. The layout was marked using tape, pegs and ropes. The plots measured 4m in length and 2m in width. Spacing between bags was 1.5m following the procedure of Reminson *et al.* (1980). There were five treatments (200ml, 400ml, 600ml, 800ml and Control) with three (3) replicates. There were each having five bags 5kg of poultry manure was added to each bag in each treatment, the addition of poultry manure acts as an inorganic fertilizer to stimulate growth of plant in the contaminated soil. The cowpea seeds were subjected to viability test using floatation technique. 2 seeds of cowpea were planted in each bag. The experimental area was hoe-weeded regularly before maturity to prevent weeds from growing around the experimental area following the procedure of Awe (2008).
The experiment was laid down in a Randomized Complete Block Design (RCBD), with three replications. Data collected was subjected to statistical analysis using Analysis of variance (ANOVA) at 5% probability level according to Agbogidi and Ofuoku (2005) and significant means where separated using Least Significant Difference (LSD).

Plate 1: Growth of cowpea at 14DAP (From left to right: 200ml, 400ml, 600ml, 800ml and control respectively)

Physico-chemical Analysis

The analysis performed include Total Petroleum Hydrocarbon (TPH), Total Sulphate, Total Nitrogen, Total Phosphorus, Total Potassuim, pH and Electrical Conductivity analysis of the soil.

Sediment pH

Procedure for pH measurement of soil was carried out in accordance with standard methods for the examination of water and wastewater (Andrew *et al*., 2005).

The pH value of sediment is measured in 1:1 sediment to water (w/v) ratio. Ten grams of air-dried sediment in 10 ml deionized water was stirred vigorously to form a thin paste and stood for an hour before pH measurement. The pH was measured using standard pH meter (Thermo Orion 9103, USA). This was measured to determine the level of acidity or alkalinity of the soil.

Electrical Conductivity (EC) Procedure for measurement of E.C of soil was carried out in accordance with standard methods for the examination of water and wastewater (Andrew *et al*., 2005). The soil sample was saturated with distilled water and mixed to a paste consistency. After standing for 1hour, the salts were dissolved and the electrical conductivity of water extracted from the paste was measured using electrodes. Calibration of the electrodes with a standard solution was necessary before measuring conductivity of the sample to ensure that the results obtained were accurate. This was measured to determine the amount of dissolved materials in the soil that will help in plant growth.

Total nitrogen.

Procedure for Total nitrogen of soil was carried out in accordance with standard methods for the examination of water and wastewater (Andrew *et al*., 2005). Five grams of air-dried sediment was shaken with 1M $NaHCO_3$ + 0.05M HCl for about 30 mins on a horizontal shaker. The extract was filtered through Whatman No. 42 filter paper and then measured by Spectrophotometer at a wavelength of 420nm. This was measured to know the levels of nitrogen in the soil because nitrogen and its compounds act as fertilizers that plants require to grow.

Total Phosphorus (TP)

Procedure for measurement of Total phosphorus of soil was carried out in accordance with standard methods for the examination of water and wastewater (Andrew *et al*., 2005).Five grams of air dried sediment was shaken with 0.5 M NaHCO3 + 0.05M HCl in for 30 mins on a horizontal shaker. The extract was filtered through Whatman No. 42 filter paper and measured by Spectrophotometer at a wavelength of 880nm. This is measured to know the levels of phosphorus in the soil because phosphorus and its compounds act as fertilizers that plants require to grow.

Total Potassium

Procedure for measurement of Total Potassium of soil was carried out in accordance with standard methods for the examination of water and wastewater (Andrew *et al*., 2005). 5g of air-dried soil sample was placed in an Erlenmeyer flask and 20ml of the extracting solution (0.05N HCl + 0.5 M NaHCO3) is added to it. Then it was placed in a horizontal stirrer and stirred for 30minutes. The resulting solution was filtered through a Whatman No. 42 filter paper into a 50ml polypropylene vial . The analytical reagent blanks was also prepared and these contained only the acid. The sample is now ready for analysis in the PerkinElmer Analyst 800 Atomic Absorption Spectrophotometer. This is measured to know the levels of potassium in the soil because potassium and its compounds act as fertilizers that plants require to grow.

Total Petroleum Hydrocarbon(TPH) in soil and plant tissue.

Procedure for measurement of TPH of soil was carried out in accordance with standard methods for the examination of water and wastewater (Andrew *et al*., 2005).10g of air-dried soil was weighed into 50ml extraction bottle, 5g sodium sulfate was added to absorb water. 20ml of Freon-113 (an extracting solvent) was added to the mixture. This mixture was kept in a sealed glass vial and placed in a sonic bath for about 30mins for assisting and hastening the extraction process. Silica gel was added to the mixture to absorb polar hydrocarbons and the mixture was mixed well. The filtered extract was then measured in a gas chromatograph. This was measured to determine the concentration of crude oil in the plant.

Physico-Chemical Parameters;

Effects of concentration on the physico-chemical parameters of the plant tissue under different levels of crude oil pollution is presented Thus
pH: The pH of the soils were not significant at ($P \leq 0.05$) with the control having slightly acidic pH. The highest pH value was observed in the 400ml concentration with pH value of 5.950 while the least pH value was recorded in the 800ml concentration (5.600).
Electrical Conductivity:
The electrical conductivity of the soil was highly significant ($P \leq 0.05$). It was observed that the electrical conductivity grew higher with low concentration of crude oil pollution. The control that lowest electrical conductivity suggesting that the crude oil in the soil enhances the electrical conductivity of that soil. The 200ml concentration had the highest value (278.8µS) while the control had the lowest value (73.3µS).
$PO^{3-}{}_4$:
This parameter was highly significant ($P \leq 0.05$). The $PO^{3-}{}_4$ was seen to decrease with increase in the level of pollution when compared with the control which recorded the lowest value (5.9mg/l) and the 200ml concentration had the highest value (80.5mg/l) as shown in Table 4.2.

P_2O_5: This parameter was highly significant ($P \leq 0.05$). The soil polluted with 600ml had the highest value of P_2O_5 (36.7mg/l) and the control maintained the least value (2.6mg/l)
PO_4: This parameter was significant ($P \leq 0.05$) when compared with the control. The highest value was observed in 600ml pollution (102.9mg/l) while the lowest value was observed in the control (10.0mg/l) It therefore follows that crude oil pollution of soil significantly increases the level of phosphate in the soil as shown in Table 4.2.
SO_4^{3-}: There was a highly significant increase ($P \leq 0.05$) in Sulphate. The 200ml had the highest value, (115.5mg/l) while the 600ml had the least value (14.5mg/l). It thus holds that SO_4^{3-} in the soil increased even at minimal level of crude oil pollution.
NO_3^-: This shows a highly significant ($P \leq 0.05$) increase with the control when compared with the level of crude oil pollution of the soil. The 800ml level recorded the highest values (154.4mg/l) followed by 200ml, 400ml, 600mls and the control had the least value (52.9mg/l)
NO_3-N: There was an increase in the NO_3-N in the soil following the highly significant difference observed when the data was analyzed at ($P \leq 0.05$). The 800ml maintained the highest value (43.6mg/l) followed by 200ml, 400ml and 600ml with the control having the least value (18.1mg/l).
Potassium (K): There was no significant increase in the level of potassium in the soil polluted by different levels of crude oil when the data was analyzed at ($P \leq 0.05$) as shown in Table 4.2. The control had the highest value (251.7mg/l) while the 400ml pollution had the lowest value (165.9mg/l). A significant difference was observed between the treatments.

Table 2: Physico-Chemical Parameters of *Vigna unguculata* Evaluated in Soil With Different Levels of Crude Oil Pollution.

	pH	E.CON	PO_4^{3-}	$P_2O_{5\,ph}$	Phos	SO_3^{4-}	NO_3^-	NO_3-N	K
200	5.742 NS	278.8 *	80.5 *	33.1 *	100.3 *	115.5 *	126.6 *	38.2 *	189.3 NS
400	5.950 NS	200.3 *	64.9 *	26.1 *	85.7 *	19.9 *	127.4 *	36.9 *	165.9 NS
600	5.708 NS	214.3 *	74.4 *	36.7 *	102.9 *	14.5 NS	115.1 *	33.3 *	228.8 *
800	5.600 NS	193.6 *	63.3 *	29.7 *	73.4 *	100.4 *	154.4 *	43.6 *	234.5 *
CONTROL	5.775 NS	73.3 *	5.9 *	2.6 *	10.0 *	69.7 *	52.9 *	18.1 *	251.7 *
LSD0.05	0.4134	29.59	28.81	12.76	37.98	54.61	36.72	9.55	34.71

TPH of Soil

There was a highly significance difference ($P \leq 0.05$) in the total petroleum hydrocarbon of the soil. The control had the least (11mg/l) while the highest value was recorded in the 800ml pollution (14077mg/l). The level of petroleum hydrocarbon found in the soil is relative to the concentration of the crude oil used in treating the soil. This shows that as the level of crude oil in the soil increases, the mean value of hydrocarbons also increases.

TPH of Plant

There was a highly significant difference ($P \leq 0.05$) in the total petroleum hydrocarbon taken up by the plant from the soil. The plants grown under the 600ml pollution was found to have the highest uptake of crude oil (3261mg/l). It was noticed that as the concentration of crude oil increased in the soil, the higher the uptake of hydrocarbon by the plant except for the treatment with 800ml. The least mean value was seen in 200ml pollution (482mg/l) presented in Table 3.

Table 3: Mean Values of Total Petroleum Hydrocarbon of Soil and Plant in Mg/l

Concentration	TPH. S.	TPH. P.
200	7991*	482*
400	12692*	499*
600	13779*	3261*
800	14077*	1607*
Control	19896	2679*
LSD0.05	5423.4	1203.8

KEY: NS=not significant, *=significantly diff.

Table 4: Mean Values of TPH of Soil in Mg/l

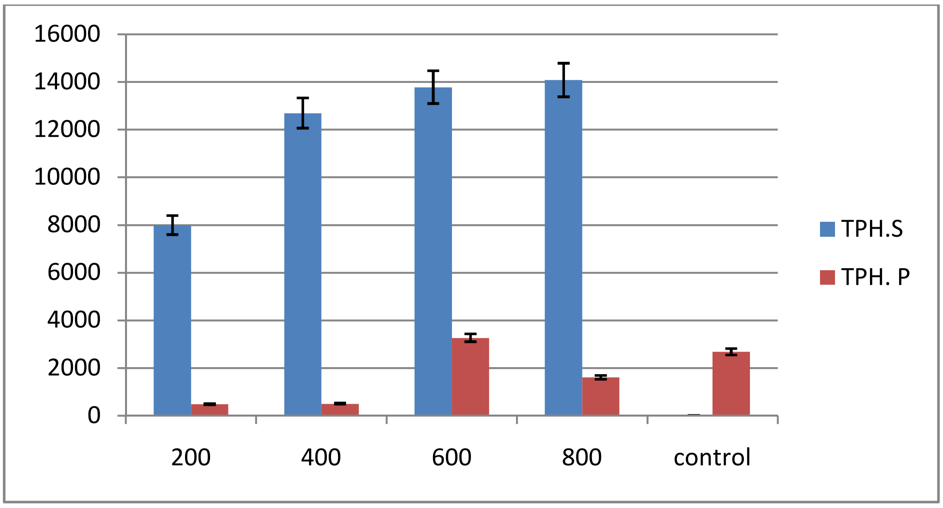
Weeks	Baseline	Week 3	Week 6	Week 9
	19896.	13533.	4509.	903.

Table 5: Mean Values of TPH of Plant in Mg/l.

Weeks	Week 3	Week 6	Week 9
	1540.	1565.	2012.

Baseline Data of mean values of TPH of soil in Mg/l/weeks.

WK 1	Soil in Mg/l wk
WK 3	3
WK 6	6
WK 9	9



EFFECT OF TPH ON SOIL AND PLANT TISSUE

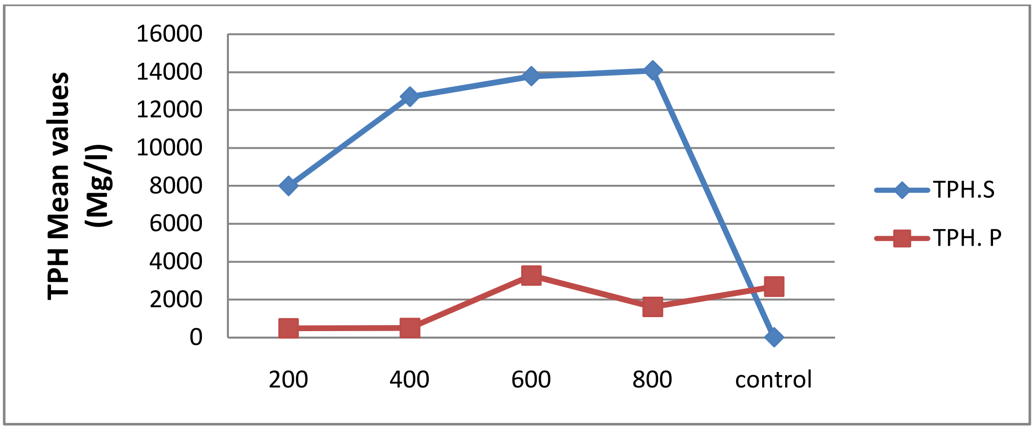


Figure 3: Effect of TPH on Soil and Plant

DISCUSSION

The result showed that *Vigna unguiculata* absorbed the hydrocarbon contaminants as there was significant reduction of the hydrocarbons by *Vigna uguculata.* As the plant grew over weeks, the level of the hydrocarbon in the soil decreased while hydrocarbon concentration increased in the plant tissue. The mean uptake rate of TPH by the plant ranged from 1540mg/l to 2012mg/l in contaminated soil after remediation. The 200ml pollution had the lowest uptake rate of 482mg/l while the 600ml pollution had the highest uptake rate of 3261mg/l. The TPH of soil reduced from 19896mg/kg recorded in the baseline data to 903mg/l recorded after 9 weeks of remediation. Though, there was a significant reduction of TPH in the soil, there was little uptake of the crude oil in the plant. Thus, the heavy reduction of TPH in the soil may also be due to the activities of microorganisms that used the contaminant as a source of carbon and energy. This corroborates with the work of (Rethy *et al.,* 1996; Quiet *et al.,* 1997).

The enhanced degradation of TPH observed agrees with the results of Soleimani *et al.,* (2010) who also observed a degradation of 64%-72% in 4700mg in polluted soil remediation by legumes TPHs per kg of soil using Tall Fescue. This may be brought about by a combination of interactions of plant and soil with microorganisms that are hydrocarbon degraders. The reduction in TPH by a may be microbial activity and increase in contact between rhizosphere microbes. It may also be due to volatilization which is the evaporation of the hydrocarbon into the atmosphere where air currents disperse the contaminants, thereby reducing their concentration. This agrees with the findings of Donaldson *et al.* (1990) who reported that only 32 days were required 30% of petroleum hydrocarbon to evaporate. Also, Dominguez Laseca *et al.* (1990) found that 64% of gasoline evaporated from beach sand after 25 min.

The plant height was significantly reduced by high level of pollution in this study. This could be attributed to deficiency in availability of nutrients needed to maintain physiological processes involved in plant growth, occasioned by nutrient stress due to influence of crude oil. This finding agrees with the works of (Ogbuehi and Ezeibekwe, 2010) who reported that crude oil because deficiency of available nutrients needed to maintain growth especially at apical regions of the crops. These reductions on growth due to high level of pollution are in agreement with findings of Molina Baharahoma *et al*. (2005) who recorded similar results and inferred that the negative effect could be due to impermeability effect of petroleum hydrocarbons or immobilization of nutrients mainly nitrogen or inhibitory effect of some polycyclic aromatic compounds.

The poor growth observed in treated plots compared to control could be partially due to the assimilation of heavy metals which were present in high toxic levels and partially to the inability of the plants in the polluted medium to absorb the nutrient from the soil possibly due to poor insulation and poor functioning of vascular bundles (phloem and xylem) (Edem *et al*., 2009).

Consequently, the Number of leaves produced by the cowpea plants was severely influenced by the concentration of crude oil. It was observed that as level of pollution increases, the number of leaves decreased (Jung 2008). This could cause by reduction in available macro and micro elements needed for production of leaves. Also it could be due to the presence of heavy metals and polycyclic aromatic compounds found in the crude oil which could cause distortion within the plant tissues (Jung, 2008).
Leaf is a site of photosynthetic activities. Crude oil pollution also caused significant reduction in leaf length and leaf width as the intensity of crude oil increased. The reduction in leaf length and leaf width will bring about a consequent reduction in surface area of leaf available for photosynthesis hence reducing photosynthetic activites (Smith *et al*., 1989).
Physicochemical Parameters;
The results on the physicochemical parameters showed that crude oil in soil caused a significant increase on physicochemical properties of the soil. These include nitrogen, phosphorus, potassium, sulphate. This observation is in harmony with earlier reports of Agbogidi and Egbuchua (2010) both noted that oil in soil has observable effects on the biological, chemical and physical properties of the soil depending on the dose, type of oil and other factors. Also, according to Agbogidi *et al*., (2006). The increase in nitrogen is justifiable since *Vigna unguculata* (a legume) is capable of fixing nitrogen in the soil by symbiotic association with *Rhizobium* (a bacterium). Thus the plant (*Vigna* sp) supplies the available nitrogen at contaminated sites. An increase in crude oil concentration increased the soil's ionic strength thereby increasing the nutrient available in the soil (Siddiqui and Adams, 2002).
Contamination of the soil had no significant effect on the pH values since no significant differences existed in pH of contaminated soil and control but the observed 5.755 value of the pH of the soil indicates that the soil is slightly acidic and this can be attributed to the high rainfall prevalent in the area leading to leaching of the basic cations from the surface area of the soil. The observed change in Electrical Conductivity when compared with the control indicates that the application of crude oil affected the ionic stability of the soil which could have contributed to the decreased conductivity with increasing oil levels (Siddiqui and Adams, 2002).
Findings support show that cowpea can contribute to the removal of pollutants from soil. Gunther *et al.* (1996) asserts to the fact that direct interaction of plant roots with hydrocarbons in the soil by sorption, uptake and transport results in the reduction of Hydrocarbons in the soil. The *Vigna unguculata* plants used in this study recorded some contaminants levels in them. This shows that *Vigna unguculata* can be used to clean up oil polluted agricultural land. (Nada *et al.,* 1995).

CONCLUSION

Phytoremediation has been recognized as a suitable tool to restore contaminated sites. The results showed that *Vigna unguiculata* differed significantly ($P \leq 0.05$) when compared with the control. This fact manifested in the plants being able to drastically reduce the high levels of hydrocarbon contaminants in the soils to very minimal levels over time. Augmentation of soil with poultry manure was also beneficial in creating the optimum conditions for the plants to grow, thereby making phytoremediation a success. There was significant degradation of hydrocarbon contaminated soil with the nutrient addition. However, *Vigna unguiculata* was able to tolerate the crude oil pollution at a concentration level of 600ml pollution because at 800ml pollution, the uptake was very minimal. This is an indication that at higher levels of crude oil pollution, the activity of *Vigna unguculata* becomes minimal. Thus, *Vigna unguiculata* can be recommended for phyto-remediation of crude oil polluted sites at low concentrations.

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