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Research article

REMEDIATION OF CRUDE OIL POLLUTED SOIL USING COW DUNG MANURE IN RELATIONS TO THE GROWTH OF MAIZE (*Zea mays L.*)

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Abstract

Simulation experiment studies were carried out on the remediation of crude oil polluted soils using cow dung manure. The natural soil sample was collected at random from the back of School of Agriculture Annex building within the Federal University of Technology Akure and was artificially spiked with 200ml Forcados Crude per 2kg soil samples, amended by 400g, 500g and 600g of cow dung manure per experimental pot and studied. Natural soil, simulated soil and treated soil samples were all characterized for pH, K, Na, Ca, Mg, cations exchange capacity (CEC), moisture, water holding capacity (WHC), porosity electrical conductivity, total organic carbon and matter, total nitrogen and phosphorus, soil particle size analysis and metals (Cu, Pb, Ni, V and Mn) using standard analytical methods to determine the effect of crude oil pollution on these properties. Results revealed that increase in cow dung manure application in the amended soils led to increase in soil minerals and nutrients. Maize plant growth parameters grown on the natural, simulated and amended soils samples were repeatedly carried out. Better growth performance of maize plant in amended soils was observed. Crude oil did affect the levels of the metals in the soil which might be due to the influence of reservoir rocks that brought slow release of heavy metals into the crude oil. Total petroleum hydrocarbon (TPH) was determined by measuring the amount of TPH left in the soil at weekly intervals in nine weeks of crude oil treatments and amendment in order to establish the effectiveness of the bioremediation process. Results showed remarkable reduction of total petroleum hydrocarbon in amended soil samples which revealed the effectiveness of cow dung manure in biodegrading petroleum hydrocarbons in the crude oil polluted soil with improved soil nutrients that enhances better crops productivity.

Keywords: Remediation, crude oil, total petroleum hydrocarbon, simulation.

Introduction

The petroleum industry has created economic boom for solving socio-economic problems [1] for Nigeria. About 30,000 barrels of crude oil spilled over 25 hectares of farmland and fresh water swamps at one location (Oyakema) alone in May 1980 [2]. An impact study of the spill in 1989 showed that it would take 8 and 33 years to clean up the topsoil and subsoil, respectively, by natural biodegradation [3]. Exploration and production of crude oil were concentrated in the Oil rich Niger-Delta region of Nigeria. Within the Niger-Delta, the numerous oil fields, tank farms, flow stations, pipelines, tankers and loading jetties constantly provide potential sources of oil pollution [4] [5]. The spilled oil pollutes soils and makes the soils to be less useful for agricultural activities with soil dependent microorganisms being adversely affected [6] [7] [8]. The socio-economic and agricultural problems caused by oil pollution to the environment most especially, in petroleum rich communities in Niger Delta region of Nigeria have led to serious un-abated food insecurity in the region. Among the factors responsible for the decrease in the productivity of the crops in Nigeria, crude oil pollution of arable soil is considered to be the major one [9]. Among cereals, maize is an important food and feed crop which ranks third after wheat and rice in the world [10]. It is recognized as a leading commercial crop of great agro-economic value owing to its expanded use in agro-industries [10], in addition to being a staple food crop for the common man in Nigeria. With these food security advantages of maize crop, the massive commercial plantations, production and storage of the crop could be used as viable tools to solve the problems of food insecurity and poverty among Nigerians. Considering the enormous agronomic and socio-economic problems that crude oil pollution could cause, it is necessary to devise an economic friendly and environmentally safe method and technique for proper clean-up of the polluted soil. The recent method is the use of microorganisms through the application of organic manure process called bioremediation [11]. The degree of soil decontamination using bioremediation techniques depend largely on the nature and levels of heavy metals and petroleum hydrocarbons present in the soil [12] [13] [14] [15]. Considering the majority that are less privileged in oil-producing region, biostimulation and biodegradation of the oil-polluted soil using cow dung manure as adopted in this study as a remediating agent could be used by farmers due to its availability, cost effectiveness, safety to the ecosystem and environment to amend the soil for human and agricultural purposes.

Materials and Method

Samples Collection: Soil samples were collected at random from the fallow land at back of School of Agriculture and Agricultural Technology (SAAT) Annex building within the Federal University of Technology Akure using a hand trowel at a depth of 0-20cm below soil surface, having no pollution history and devoid of hydrocarbon contamination. Crude oil with specific gravity of 0.75g/cm^3 was obtained from Shell Petroleum Development Company (SPDC) forcados terminal, Burutu, Delta state, Nigeria. Maize seeds were purchased at Oba market in Akure, Ondo State, Nigeria. Cow dung was collected from the Teaching and Research Farm (Livestock section) of the Federal University of Technology, Akure, Nigeria.

Sample Preparation, Simulation and Amendment: Soil was air dried for a period of one week in a clean well ventilated laboratory and sieved by passing through a 2mm mesh sieve. 2kg of soil was each measured into clean dry experimental planting pot and moistened with distilled water to ensure proper mixing with the crude oil. Simulation of the soil samples was done by measuring 200ml of crude oil into the experimental planting pot containers containing 2kg soil each. The individual mixtures were thoroughly mixed to achieve a 10% artificial pollution. 10% spiking was adopted to achieve severe pollution because beyond 3% concentration [16], crude oil has been reported to be increasingly deleterious to soil biota and crop growth [17]. The cow dung manure sample was sun dried for one week after which it was grinded, thoroughly mixed, sieved through a 2mm sieve to achieve uniform particle size and stored in neat polythene bag for use. In variations, 400g, 500g and 600g of the cow dung manure were added to the experimental planting pot containing 2kg of crude oil simulated soil in ratio (1:2:10), (1:2.5:10), (1:3:10) respectively and thoroughly mixed to obtain homogeneity and to allow proper decomposition for another one week with constant watering before planting the maize seeds. Then, two healthy seeds of maize were planted per experimental planting pot. The experimental planting pot containing 200ml of crude oil simulated soil served as the (control 2) and experimental planting pot containing 2kg of natural soil as (control 1) in ratio (1:0:10) and (0:0:10) respectively. This experimental design was a randomized complete block and duplicated. Plants height, leaf area and number of tillers were measured weekly until after nine weeks of planting.

Soil Characterization/Physicochemical Analysis: Soil physicochemical characteristics such as soil particle size analysis, pH, K, Na, Ca, Mg, cations exchange capacity (CEC), moisture, water holding capacity (WHC), porosity, electrical conductivity, total organic carbon, organic matter, total nitrogen, phosphorus and heavy metals (Cu, Pb, Ni, V and Mn) were determined before pollution, nine weeks after pollution and bioremediation process. Soil pH was determined electrometrically following the procedure outlined by Mylavarapus and Kennelley

[18]. Particle size analysis was done using bouyoucos hydrometer method [19]. Soil minerals were determined by the method of Tel [20]. Total organic carbon and matter were determined by the wet dichromate acid oxidation method Nelson and Sommers [21]. Soil water holding capacity and porosity were determined by the method of Michael [22]. Total Nitrogen was determined using the method of Radojevic and Bashkin [23]. Total Phosphorus was determined by Bray and Kurtz method Bray and Kurtz method [24]. Electrical conductivity was carried out as described by Chopra and Kanzer [25]. Soil moisture was determined using the method Michael [22]. Heavy metals were determined by digesting the samples with concentrated mixtures of hydrofluoric, nitric and perchloric acid AOAC [26] and analyzed by the atomic absorption spectrophotometer.

Determination of maize plant growth: These were determined by measuring the plant height, number of tillers, leaf area and biomass (root, stem and leaves) of the maize plant in each treatment. The plant height was measured with tape rule while the dry weight biomass of the plant was determined by measuring the dry matter content of the plant after washing with distilled water, sun drying and oven drying the plant (root, stem and leaves) in an oven at 60^{o}C for 48 hours to a constant weight using a weighing balance [27]. Numbers of tillers were determined by counting. The leaf areas of the plant were measured following the method described by Eze, [28].

Determination of Total petroleum Hydrocarbon (TPH): 1g of the simulated and amended soil samples were dissolved in 10ml of hexane and shaken for ten minutes using a mechanical shaker. The solution was filtered using a whatman filter paper and the filtrate diluted by taking 1ml of the extract into 50ml of hexane [16]. Procedural blanks and standard solutions were prepared and included to ensure analytical quality control so as to assure the accuracy and reproducibility of the results. The absorbance of this solution was read at 460nm with HACH DR/2010 Spectrophotometer using n-hexane as blank. Replicate analyses were carried out on the determination of TPH to yield a mean which will be used to determine trueness and also standard deviation of the mean to measure precision Stanton [29], Valcarcel [30]. Total petroleum hydrocarbon (TPH) was determined at weekly intervals for nine weeks.

Results and Discussion

Pollution of soil with crude oil has caused the reduction of soil pH, Conductivity, N, P, K^+, Na^+, Ca^{2+}, Mg^{2+}, CEC, Moisture, WHC and Porosity from 6.05 to 4.46, 219.47μs/cm to 161.79μs/cm, 0.22% to 0.21%, 21.55% to 17.87%, 0.29 Cmol/kg to 0.22 Cmol/kg, 0.20 Cmol/kg to 0.18 Cmol/kg, 2.30 Cmol/kg to 2.00 Cmol/kg, 2.00 Cmol/kg to 1.70 Cmol/kg, 4.79 Cmol/kg to 4.12 Cmol/kg, 10.13% to 10.06%, 37.01% to 36.89% and 46.48% to 46.32% respectively. The observed reduction in pH and conductivity was in line with the findings of Osuji and Nwoye [31]. This could be as a result of increase in hydrophobicity of the soil condition which prevented air and water to penetrate the contaminated soil that favoured the release of hydrogen ion (H^+) that made the soil increase in acidic condition as crude oil application increases. Reduced conductivity could be due to the non-polar nature of the crude oil bringing about reduced ionic movement in the soil [16]. The reduction in the values of N, P, K^{+}, Na^{+}, Ca^{2+}, Mg^{2+}, CEC, Moisture, WHC and Porosity in crude oil polluted soil might be due to the immobilization of these soil nutrients and minerals by crude oil. Crude oil will make the soil hydrophobic and make less water and air available for the nutrients and minerals to be dissolved and available in aqueous phase of the soil in solution. However, more nutrients and minerals must have been trapped in organic phase of the crude oil contaminated soil and un-available to the soil in solution. Crude oil in soil makes the soil condition un-satisfactory for plants [32], due to the reduction in the level of available plant nutrients or a rise in toxic levels of certain element such as iron and zinc [33]. However, the organic carbon, organic matter and C/N increased in crude oil polluted soil from 1.57% to 1.63%, 2.71% to 2.81% and 7.14 Ratio to 7.76 Ratio respectively. Similar results have been reported by [34]. The organic carbon and organic matter increase may be due to the added carbon substrate from the crude oil. The increase in C/N Ratio was an indication of stress caused by crude oil contamination to the soil. The observed increase in pH from (6.71 to 6.74 to 6.85) and conductivity (243.41 to 244.50 to 248.49 µs/cm) with increase in cow dung manure during the bioremediation process was due to the additional nutrients supplement being supplied by cow dung manure like calcium, magnesium, sodium, phosphorus, potassium and nitrogenous nutrients that microbially mineralized the amended soil which have contributed to the improved soil properties. This is in agreement with the findings of Akpoveta, *et al* [16] and Urunmatsoma, *et al* [35]. Soil properties such as total nitrogen (0.41 to 0.55 to 0.95%), phosphorus (27.01 to 28.53 to 32.22%), organic carbon (1.61 to 1.67 to 1.70%) and organic matter (2.78 to 2.88 to 3.14%), potassium (0.32 to 0.37 to 0.44 Cmol/kg), sodium (0.29 to 0.30 to 0.33 Cmol/kg), calcium (3.00 to 3.28 to 3.30 Cmol/kg), magnesium (2.01 to 2.10 to 2.40 Cmol/kg) and CEC (5.62 to 6.05 to 6.47) increased respectively with increase in cow dung manure during the bioremediation process as seen in table 1. The more the cow dung manure being added, the more the nutrients, minerals and more petroleum utilizing microorganisms being supplied by cow dung manure population available for soil mineralization [36]. Increase in organic carbon and organic matter in amended soil might be due to the increase in humification of organic materials of the cow dung manure with increase in cow dung manure [37]. The reduction in C/N in amended planting pots with increase in cow dung manure from (3.93 to 3.04 to 1.79) in compared with natural (7.14) and simulated planting pots (7.76) was due to the microbial fixation of nitrogen from atmosphere into the amended soil with the added nutrients from cow dung and the microbial utilization of the crude oil that favoured microbial cell development, biomass increase and populations [38]. Crude oil did not negatively affect these soil properties as seen from the results. Particle size analysis shows that the sand (45, 45, 45, 45, and 45%), clay (33, 33, 33, 33, and 33%) and silt (22, 21, 21, 22, and 22%) fractions respectively were all in the same range for the natural, simulated and amended soils. A classification of the soil based on the USDA textural class [39] shows that the soil is Sandy clay loam. This shows that there was no effect on the soil texture. The concentrations of copper, lead, nickel, vanadium and manganese in the natural soil were found to be 0.021mg/kg, 0.004mg/kg, 0.027mg/kg, 0.003mg/kg and 0.011mg/kg respectively, but gave higher concentrations of 0.141mg/kg, 0.283mg/kg, 0.176mg/kg, 0.074mg/kg and 0.220mg/kg in the crude oil simulated soil, suggesting hydrocarbon influence on the metals. This might be due to the influence of reservoir rocks that brought slow release of heavy metals into the crude oil simulated soil solution which depends on strong depletion of minerals content of the soil solution, decomposition, oxidation of organic matter that could be released at low pH [35]. However, the concentrations of copper, lead, nickel, vanadium and manganese were found to increase with increase in cow dung manure in amended planting pots from (0.144 to 0.149 to 0.203mg/kg), (0.291 to 0.297 to 0.321mg/kg), (0.177 to 0.079 to 0.084mg/kg), (0.076 to 0.076 to 0.079mg/kg) and (0.223 to 0.228 to 0.235mg/kg) respectively. The increase in the values of pH, soil minerals, nutrients and organic matter at 400g, 500g and 600g of the cow dung manure amendment level did more to bind the metals than make them available for plant uptake [35]. In general, the concentration of an element in the soil solution is believed to depend on the equilibrium between the soil solution and solid phase, with pH playing the decisive role [40]. The soil's ability to immobilize heavy metals increases with rising pH and peaks under mild alkaline conditions [35]. The concentration of the metals in the natural soil simulated and amended soils before and after the treatment were within the WHO/FAO permissible and guidelines for safe limits of heavy metals in soil [41]. Some heavy metals at low doses are essential micronutrients for plants but in higher doses may cause metabolic disorders and growth inhibition for most of the plant species [42].

Table 1: Results of nutrient analysis; soil physicochemical properties and heavy metals before, nine week after simulation, remediation and planting (9WAP)

Parameters	Crude oil sample	Cow dung manure	2kg of natural soil (control 1)	2kg of soil + 200ml of crude oil (control 2)	2kg of soil + 200ml of crude oil + 400g of cow dung	2kg of soil + 200ml of crude oil + 500g of cow dung	2kg of soil + 200ml of crude oil + 600g of cow dung
pH	4.22 ±0.141	7.70 ±0.100	6.05 ±0.000	4.46 ±0.158	6.71 ±0.000	6.74 ±0.050	6.85 ±0.100
Conductivity(μ s/cm)	-	-	219.47 ±0.00	161.79 ±1.00	243.41 ±0.00	244.50 ±0.00	248.49 ±1.01
OC (%)	97.05 ±0.10	13.45 ±0.00	1.57 ±0.14	1.63 ±0.05	1.61 ±0.10	1.67 ±0.00	1.70 ±0.00
OM (%)	_	23.19	2.71	2.81	2.78	2.88	3.14
N (%)	0.10 ±0.000	1.29 ±0.010	0.22 ±0.010	0.21 ±0.024	0.41 ±0.015	0.55 ±0.014	0.95 ±0.000
P (%)	0.06 ±0.005	0.56 ±0.024	21.55 ±0.024	17.87 ±0.010	27.01 ±0.00	28.53 ±0.00	32.22 ±0.010
K^+ [Cmol/kg]	0.07 ±0.010	2.12 ±0.000	0.29 ±0.002	0.22 ±0.014	0.32 ±0.010	0.37 ±0.014	0.44 ±0.000

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Na^{+} [Cmol/kg]	0.07 ±0.000	0.98 ±0.010	0.20 ±0.000	0.18 ±0.010	0.29 ±0.000	0.30 ±0.010	0.33 ±0.010
Ca^{2+} [Cmol/kg]	0.11 ±0.016	0.20 ±0.010	2.30 ±0.024	2.00 ±0.016	3.00 ±0.000	3.28 ±0.000	3.30 ±0.016
Mg^{2+} [Cmol/kg]	0.09 ±0.010	0.51 ±0.000	2.00 ±0.010	1.70 ±0.024	2.01 ±0.016	2.10 ±0.016	2.40 ±0.000
CEC [Cmol/kg]	0.4	3.81	4.79	4.12	5.62	6.05	6.47
$^{C}/_{N}$ Ratio	97.05	10.43	7.14	7.76	3.93	3.04	1.79
Moisture (%)	-	-	10.13 ±0.100	10.06 ±0.160	10.29 ± 0.240	10.50 ±0.100	11.01 ±0.000
WHC (%)	-	-	37.01 ±0.158	36.89 ±0.141	37.16 ± 0.000	37.36 ±0.100	37.84 ±0.158
Porosity (%)	-	-	46.48 ±0.000	46.32 ±0.240	46.66 ± 0.141	46.91 ±0.156	47.52 ±0.160
Cu (mg/kg)	0.150 ±0.010	0.002 ±0.000	0.021 ±0.024	0.141 ±0.000	0.144 ±0.000	0.149 ±0.016	0.203 ±0.000
Pb (mg/kg)	0.280 ±0.000	0.170 ±0.014	0.004 ±0.000	0.283 ±0.015	0.291 ±0.010	0.297 ±0.000	0.321 ±0.024
Ni (mg/kg)	0.190 ±0.015	0.110 ±0.010	0.027 ±0.024	0.176 ±0.000	0.177 ±0.000	0.079 ±0.010	0.084 ±0.016
V (mg/kg)	0.080 ±0.016	0.102 ±0.000	0.003 ±0.000	0.074 ±0.015	0.076 ±0.000	0.076 ±0.010	0.079 ±0.014
Mn (mg/kg)	0.370 ±0.010	0.010 ±0.000	0.011 ±0.000	0.220 ±0.014	0.223 ±0.016	0.228 ±0.000	0.235 ±0.024
Sand (%)	-	-	45	45	45	45	45
Clay (%)	-	-	33	33	33	33	33
Silt (%)	-	-	22	21	21	22	22
Textural class	-	-	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam

Results = Mean values ± Standard deviation

The general growth (height, number of tillers and total leaf area) and biomass performance of maize plants planted for the period of nine weeks in simulated soils were poor in comparison with the natural soils as seen in tables 2, 3, 4 and 5 respectively. This was due to the crude oil immobilization of soil and plants nutrients, increase in hydrophobic condition of the soil which could disrupt the osmotic process of the water and nutrients, between soil and maize plants (Roots) [43], exhaustion of limited soil nutrients by the microbes, poor wetability and poor aeration to the general growth variation. However, the general growth (height, number of tillers and total leaf area) and biomass performance of maize plants planted for the period of nine weeks were increase with the increase (400g, 500g and 600g cow dung manure) respectively in cow dung manure amended planting pots. This was due to the general growth variations observed in tables 2, 3, 4 and 5 which was attributed to the nutrients supplement addition and petroleum utilizing microorganisms supplied by cow dung manure that utilized the crude oil for microbial cell development, microbial population and microbial biomass to mineralize the cow dung manure and subsequently improved the physico-chemical, nutrients and minerals of the amended soil which reflected on the general growth and biomass performance of maize plants [44]. Using organic manure like cow dung as an organic soil amendment stimulates microorganisms to take nitrogen from air and fix it into the soil where plants can use it and also a plant rich in decomposing organic matter provides a much higher level of CO_2 in the air just above the soil for the plant use [35]. Plants have been reported by Onuh *et al.*, [45] to grow better with adequate soil nutrients even in the face of crude oil pollution which was also observed in this research work.

Table 2: Effects of cow dung on maize plants height (cm), planted on crude oil polluted soil

Weeks After Planting	2kg of natural soil (control 1)	2kg of soil + 0g of cow dung + 200ml of crude oil (control 2)	2kg of soil + 400g of cow dung + 200ml of crude oil	2kg of soil + 500g of cow dung + 200ml of crude oil	2kg of soil + 600g of cow dung + 200ml of crude oil
1	13.00 ±1.83	11.25 ±1.85	14.00 ±2.71	18.38 ±1.89	20.38 ±1.13
2	22.00 ±0.82	19.25 ±2.60	27.00 ±2.71	32.75 ±2.22	35.25 ±1.71
3	38.50 ±2.35	32.00 ±2.45	46.25 ±0.96	53.00 ±2.12	56.38 ±2.21
4	57.50 ±2.52	39.50 ±1.68	66.50 ±1.29	75.00 ±1.15	77.50 ±1.73
5	77.00 ±1.63	47.13 ±0.25	84.25 ±1.76	98.75 ±1.44	101.00 ±1.73
6	81.88 ±1.03	55.00 ±1.41	104.50 ±1.29	121.00 ±2.58	123.75 ±1.50
7	97.75 ±0.50	61.88 ±1.44	122.13 ±1.03	144.75 ±2.21	158.75 ±1.50
8	111.25 ±0.96	69.25 ±1.89	146.25 ±0.96	175.00 ±1.15	179.00 ±0.82
9	133.25 ±0.96	88.25 ±1.50	150.00 ±0.82	160.00 ±0.83	203.75 ±1.26

Results = Mean values ± Standard deviation

Table 3: Effects of cow dung on number of tillers of maize plants, planted on crude oil polluted soil

Weeks After Planting	2kg of natural soil (control 1)	2kg of soil + 0g of cow dung + 200ml of crude oil (control 2)	2kg of soil + 400g of cow dung + 200ml of crude oil	2kg of soil + 500g of cow dung + 200ml of crude oil	2kg of soil + 600g of cow dung + 200ml of crude oil
1	4.00 ±0.00	3.75 ±0.50	4.50 ±0.58	4.50 ±0.58	5.00 ±0.00
2	5.25 ±0.50	4.50 ±0.58	6.25 ±0.50	7.25 ±0.50	7.50 ±0.58
3	7.00 ±0.00	5.75 ±0.50	8.25 ±0.50	8.75 ±0.50	9.75 ±0.50
4	10.25 ±0.50	6.00 ±0.00	11.50±0.58	12.50 ±0.58	13.75 ±0.50

5	11.00 +0.00	6.75 +0.50	12.75 +0.50	14.00 +0.00	15.50 +0.58
6	12.25 +0.50	7.25 +0.50	13.25 +0.50	14.75 +0.50	16.50 +0.58
7	12.75 +0.50	8.75 +0.50	14.75 +0.50	16.75 +0.50	17.75 +0.50
8	13.25 +0.50	10.25 +0.50	15.75 +0.50	17.50 +0.58	18.25 +0.50
9	13.75 +0.50	10.75 +0.50	16.75 +0.50	18.75 +0.50	19.50 +0.58

Results = Mean values + Standard deviation

Table 4: Effects of cow dung on total leaf area (cm^3) of maize plants, planted on crude oil polluted soil (9WAP)

STUDY	2kg of natural soil (control 1)	2kg of soil + 0g of cow dung + 200ml of crude oil (control 2)	2kg of soil + 400g of cow dung + 200ml of crude oil	2kg of soil + 500g of cow dung + 200ml of crude oil	2kg of soil + 600g of cow dung + 200ml of crude oil
Total Leaf Area	5.95 +0.06	3.53 +0.01	8.23 +0.02	11.22 +0.01	13.74 +0.03

Results = Mean values + Standard deviation; 9WAP = Nine week after planting

Table 5: Effects of cow dung on maize plant biomass (g) on crude oil polluted soil (9WAP)

Maize plant organs	2kg of natural soil (control 1)	2kg of soil + 0g of cow dung + 200ml of crude oil (control 2)	2kg of soil + 400g of cow dung + 200ml of crude oil	2kg of soil + 500g of cow dung + 200ml of crude oil	2kg of soil + 600g of cow dung + 200ml of crude oil
Roots	1.00 +0.01	0.87 +0.02	4.17 +0.02	5.00 +0.04	5.80 +0.02
Stems	1.85 +0.01	0.83 +0.02	10.10 +0.02	10.82 +0.04	13.82 +0.02
Leaves	4.62 +0.01	2.53 +0.02	14.52 +0.01	19.65 +0.01	20.17 +0.02
Total Biomass	7.47 +0.01	4.23 +0.01	28.79 +0.01	35.47 +0.01	39.79 +0.02

Results = Mean values + Standard deviation; 9WAP = Nine week after planting

The potential of the treatment option was shown in table 6 and 7 by the reduction (mg/kg)/ percentage reduction (%) of the crude oil in the amended soil was observed in the sample supplemented with highest cow dung manure (600g), followed by the higher treatment (500g) down to the least treatment concentration (400g) remarkably but simulated soil samples weekly until nine week after planting. The total petroleum hydrocarbons (TPH) content in simulated soil was observed in table 6 to be 1628 mg/kg in 0 week before planting. Total petroleum hydrocarbon content expectedly increased the level of crude oil in polluted soil [46]. The TPH was poorly reduced in simulated soil sample weekly until after ninth week of planting as observed (1499.39 mg/kg/7.90 %). This may be due to the microbial activities of the indigenous petroleum utilizing microbes that may have been present or found in the crude oil polluted soil [47]. The gravimetric reduction (mg/kg)/ percentage reduction (%) in ninth week (52.10

mg/kg/96.80 %) of the total petroleum hydrocarbon in amended soil of 600g cow dung manure /200ml crude oil in 2kg soil planting pots. The appreciable total petroleum hydrocarbon reduction (mg/kg)/ percentage reduction (%) being observed in other amended planting pots in ninth week (95.89 mg/kg/94.11 %) and (165.08 mg/kg/89.86 %) for 500g and 400g cow dung application respectively may be due to the increase in petroleum utilizing microbes population and biomass in cow dung manure [48] that utilized the crude oil for carbon and energy source to degrade crude oil in amended soil [49]. Organic manure like cow dung increase the rate of biodegradation of the pollutant [50] and some of the products of biodegradation are useful plants nutrients, organic matter and organic fertilizers which do not destroy beneficial microorganisms and earthworms [35].

Table 6: Concentration (mg/kg) decrease of total petroleum hydrocarbon (TPH) with time for nine weeks of the bioremediation of crude oil simulated soil and amended soil.

Weeks After Planting	2kg of soil + 0g of cow dung + 200ml of crude oil (control)	2kg of soil + 400g of cow dung + 200ml of crude oil	2kg of soil + 500g of cow dung + 200ml of crude oil	2kg of soil + 600g of cow dung + 200ml of crude oil
0	1628	1628	1628	1628
1	1566.34 ±1.00	761.74 ±1.58	723.97±1.01	703.30 ±0.00
2	1544.97 ±1.58	702.97 ±1.00	683.27 ±0.00	640.29 ±1.58
3	1523.00 ±1.01	603.99 ±1.10	567.52 ±0.00	520.63 ±0.00
4	1517.30 ±0.00	584.29 ±1.58	482.70 ±1.58	432.89 ±0.00
5	1514.04 ±1.10	531.05 ±1.20	390.56 ±1.00	307.69 ±1.20
6	1512.41 ±1.00	428.00 ±1.01	289.46 ±1.58	211.48 ±1.58
7	1509.16 ±1.10	319.58 ±0.00	206.43 ±1.20	137.73 ±1.00
8	1502.64 ±1.20	238.99 ±0.00	152.87 ±1.58	96.54 ±1.01
9	1499.39 ±1.58	165.08 ±1.00	95.89 ±1.00	52.10 ±1.00

Results = Mean values ± Standard deviation

Table 7: Percentage (%) decrease of total petroleum hydrocarbon (TPH) with time for nine weeks of the bioremediation of crude oil simulated soil and amended soil.

Weeks After Planting	2kg of soil + 0g of cow dung + 200ml of crude oil (control)	2kg of soil + 400g of cow dung + 200ml of crude oil	2kg of soil + 500g of cow dung + 200ml of crude oil	2kg of soil + 600g of cow dung + 200ml of crude oil
0	100	100	100	100
1	3.80	53.21	55.53	56.80
2	5.10	56.82	58.03	60.67
3	6.40	62.90	65.14	68.02
4	6.80	64.11	70.35	73.41
5	7.00	67.38	76.01	81.10
6	7.10	73.71	82.22	87.01
7	7.30	80.37	87.32	91.54

8	7.70	85.32	90.61	94.07
9	7.90	89.86	94.11	96.80

Results = Mean values ± Standard deviation

Conclusion

It was revealed in this study that 200ml of crude oil in 2kg capacity of natural Sandy clay loam soil rendered the physico-chemical properties of soil unsatisfactory and also found to retard the growth of maize plant grown in the soil. 600g of cow dung manure in the presence of 200ml of crude oil best improved the physico-chemical properties of 2kg amended soil. These results have shown the effectiveness of cow dung manure at degrading crude oil polluted soils and that the organic nutrient supplementation enhances the biodegradation rate. Remediation of crude oil polluted soil with cow dung manure has been established to be highly effective towards the improvement of the minerals, nutrients and physico-chemical properties of the amended soil and support maize plant growth enhancement to solve food insecurity in crude oil contaminated region in Nigeria.

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References

[1] Ibekwe V. I., Ubochi, K. C., and Ezeji, E. U., Effect of organic nutrient on microbial utilization of hydrocarbons on crude oil contamination soil, Africa Journal of biotechnology, vol. 5, (10), 2006, pp. 983- 986.

[2] Ifeadi C. N. and Nwankwo J. N., Oil spill incidents in Nigeria petroleum industry. A critical analysis, Napector, 8, 1989, 11-45.

[3] Abu O. G. and Ogiji A. P., Initial Test of a Bioremediation Scheme for the Clean up of an oil polluted water body in a rural community in Nigeria, Bio-resource Technology, 58, 1996, 7-12.

[4] Ijah U. J. J. and Antal S. P., Removal of Nigerian light crude oil in soil over a 12 month period, Int. Biodeterio. Biodeg., 51, 2003, 93-99.

[5] Chikere B. O. and Chijioke-Osuji O., Microbial diversity and physicochemical properties of a crude oil polluted soil, Niger. J. Microbiol, 20, 2006, 1039-1046.

[6] Siddiqui S. and Adams W. A., The fate of diesel hydrocarbons in soils and their effects on germination of perennial ryegrass, Environmental Toxicology, 17(1), 2002, L 49-62.

[7] Lundstedt S., Analysis of PAHs and their transformation product in contaminated soil and remedial processes, Solfjodern Offset A.B., Umea, Sweden, 2003, 55pp.

[8] Nwachukwu S. C. U. and Ugoji E. O., Impacts of crude petroleum spills on microbial communities of tropical soils, Int. J. Ecol. Environ. Sci. 21, 1995, 169-176.

[9] Onweremadu E. U. and Duruigbo C. E., Assessment of Cd concentration of crude oil polluted arable soils, International Journal of Environmental Science Technology 4(3), 2007, 409-412.

[10] Rasheed M., AH H. and Mahmood T., Impact of Nitrogen and Sulfur application in growth and yield of maize (Zea mays L.), Crop Journal of Research (Science) 15(2), 2004, 153-157.

[11] Sasikumar C. S. and Taniya P., Environmental Management Bioremediation of polluted environment, Proceeding of the third International Conference of Environment and Health, Chemia University of Madra, Chennal India, 15-17 December, 2003., pp. 465-469.

[12] Marwood M. T., Knoke K., Yau K., Lee H., Trevors J. T., Suchorski T., Flemming C. A., Hodge V., Liu D. L. and Seech A. G., Comparison of toxicity detected by five biossays during bioremediation of diesel fuel-spiked soils, Environ Toxical Water QuaL, 13, 1998, 117-29.

[13] Kelly J. J. and Tate R. E., Effects of heavy metals contamination and remediation on soil microbial communities in the vicinity of a Zn smelter, J Environ Qual, 27, 1998, 609-617.

[14] Amadi A., Abbey S. D., Nma A., Chloric effects of oil spill on soil properties and micro flora of a rainforest ecosystem in Nigeria, Water, Air Soil Pollut., 6, 1996, 1-11

[15] Hewari J., Bealie C., Oullete D., Pontbriand Y., Halsaz A. M., Vanata H., Determination of petroleum hydrocarbons in soil: SFE versus Soxhlet and water effect on recovery, Int. Journal Environ. Anal Chem, 60, 1995,123-37.

[16] Akpoveta O. Vincent, Egharevba Felix, Medjor O. Weltime, Osaro K. Ize-iyamu and Enyemike E. Daniel, Microbial Degradation and its Kinetics on Crude Oil Polluted Soil, Res.J.Chem.Sci, Vol. 1(6), Sept 2011, 8-14

[17] Osuji L.C., Egbuson E.J.G. and Ojinnaka C.M., Chemical reclamation of crude-oil- inundated soils from Niger Delta, Nigeria, Chem. Ecol., 21(1), 2005, 1-10

[18] Mylavarapu R.S. and Kennelley E.D., UF/IFAS Extension Soil Testing laboratory (ESTL), Analytical procedures and Training Manual, 2002, 350

[19] Bouyouces G.H., The Hydrometer Method for the Determination of Soil Particle Size, Agron, J., 43, 1951, 434-438

[20] Tel, D. A., Soil and plant analysis IITA, Department of Land Resource Science University of Fuclph, Guclph, Ontario, Canada, 1984, P 117-190.

[21] Nelson D.W. and Sommers L.E., Total carbon, Organic matter, In: Page, A.L et al. (Eds.), Methods of soil Analysis Part 2, Agronomy Monograph 9, 2nd Edn., American Society for Agronomy and Soil Science Society of America. Madison, Wisconsin, 1982, pp 539 – 579

[22] Micheal, A. M, Irrigation theory and practice, Pub. Bikas publishing house PUT Ltd, New Delhi, 1978

[23] Radojevic M. and Bashkin V.N., Practical environmental analysis, Royal society of chemistry, Cambridge U.K, 1999

[24] Bray R.H. and Kurtz L.T., Determination of total organic and available forms of phosphorus in soils, Soil Sci., 59, 1943, 39-45

[25] Chopra G. and Kanzar C., Analytical Agricultural chemistry, 2nd edition, Prentice-Hall, India, 1988

[26] AOAC, The Analyst Association of Analytical Chemist, 1970

[27] Merkl N., Schutze-Kraft R. and Infante C., Phytoremediation in the tropics. The effect of crude oil on the growth of tropical plants, Bioremediation Journal, 8 (3-4), 2004, 177-184.

[28] Eze J. M. O., Studies on growth regulation, salt uptake and translocation, Ph.D. Thesis, University of Durban, England, 1965, pp, 31-33.

[29] Stanton R.E., Rapid methods of Trace Analysis for Geochemical Applications, London: Edward Arnold Ltd, 1966

[30] Valcarcel M., Principles of analytical Chemisry, New York: Springer-Verlag Berlin Heidelberg, 2000

[31] Osuji L.C. and Nwoye I., An appraisal of the impact of petroleum hydrocarbons on soil fertility: the Owaza experience, Afri J of Agric Res., 2 (B), 2007, 318-324

[32] De Jong E., Effect of a crude oil spill on cereals, Environmental Pollution., 22, 1980, 187-196.

[33] Udo E. J. and Fayemi A. A. A., The effect of oil pollution on germination, growth and nutrient uptake of corn, Journal Environ, Qtial, 4, 1975, 537-540.

[34] Okolo J. C., Amadi E. N. and Odu C. T. I., Effects of Soil treatments containing poultry manure on crude oil degradation in sandy loam soil. Applied Ecology and Environmental Research, 3(1), 2005, 47-53.

[35] Urunmatsoma, S. O. P., Ikhuoria, E. U., and Okieimen, Chemical fractionation and heavy metal accumulation in maize (zea mays) grown on chromate copper arsenate (CCA) contaminated soil amended with cow dung manure, International for Biotechnology and molecular Biology Research Vol. 1(6), 2010, pp65-73.

[36] M.J. Ayotamuno, O.M. Olatunji and S.T. Ikari, Petroleum Contaminated Soil Remediation Using Poultry Feaces and Plantain Stem, International Journal of Scientific & Engineering Research Volume 4, Issue3, March-2013 ISSN 2229-5518

[37] Onyeagba, R.A; Nwaugo, V.C., Asamudu. and Nduka J.O., Effects of Pig and Chicken Wastes Composts on Physico-Chemical and Biological Indices of Petroleum Contaminated Orashi-River Wetland in Egbema Southern Nigeria, ABSU Journal of Environment, Science and Technology Volume, 1, 2011, 1-13

[38] Ibiene, A. A., Orji, F. A., Ezidi, C. O. and Ngwobia1, C. L., Bioremediation of Hydrocarbon Contaminated Soil in the Niger Delta using spent Mushroom Compost and other Organic Wastes, Nigerian Journal of Agriculture, Food and Environment. 7(3), September, 2011, 1-7

[39] United State Department for Agriculture (USDA), National Resources Conservation Service, http://websoilsurvey.nrcs.usda.gov, 2002, 2-29

[40] Lindsay W. L, Chemical equilibria in soils. John Wiley & Sons, New York, NY, 1979

[41] Lăcătuşu R., Rîşnoveanu I., Kovacsovics B., Lungu M., Pollution with heavy metals of soils in the Eastern part of Bucharest city, Ştiinţa Solului, XXXIV, 1, 2000, 121-134 (published in Romanian).

[42] Claire L. C., Adriano D. C., Sajwan K. S., Abel S. L., Thoma D. P. and Driver J. T., Effects of selected trace metals on germinating seeds of six plant species, Water, Air and Soil Pollution, 59, 1991, 231 -240.

[43] Njoku, K. L., Akinola, M. O. and Oboh, B. O., Growth and performance of Glycine max L. (Merrill) in crude oil contaminated soil augmented with cow dung. Nat. Sci. 6(1), 2008, 48-58.

[44] Merkl N., Schutze-Kraft R. and Arias M, Influence of fertilizer level on Phytoremediation of crude oil contaminated soils with the tropical grass Brachiaria brizantha (Hochst. Ex A, Rich.) Stapf. In: Phytoremediation of Petroleum- contaminated soil, Merkl, N., (Ed), Margraf Publisher, Weikershim, 2005, pp 71-83.

[45] Onuh M. O., Ohazurike H. C. and Madukwe D. K., Interaction of crude oil and manure treatments and its effects on the aquonomic characteristics of maize (Zea mays L.), Science World Journal, vol. 3 (No. 2), 2008

[46] Ogboghodo I. A., Erebor E. B., Osemwota I.O. and Isitekhale H. H., The effects of application of poultry manure to crude oil polluted soils on maize (zea mays) growth and soil properties, Environmental Monitoring and Assessment 96, 2004, 153–161

[47] Okolo J. C., Amadi E. N. and Odu C. T. I., Effects of Soil treatments containing poultry manure on crude oil degradation in sandy loam soil. Applied Ecology and Environmental Research, 3(1), 2005, 47-53.

[48] Obire O., Anyanwu E. G. and Okigbo R. N., Saprophytic and crude oil degrading fungi from cow dung and poultry droppings as bio-remediating agents, Journal of Agricultural Technology, vol. 4(2), 2008, 81-89.

[49] Ibekwe V. I., Ubochi, K. C., and Ezeji, E. U., Effect of organic nutrient on microbial utilization of hydrocarbons on crude oil contamination soil, Africa Journal of biotechnology vol. 5 (10), 2006, pp. 983- 986.

[50] William J. T. and Michael A. P., Introduction to Biotechonlogy, Second Edition, 2009, p216