Fertilizer-assisted Bioremediation of Sandy and Silty Clay Soils contaminated with Crude Oil

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Abstract

Crude oil contamination of farmlands and rivers has been a major concern in Nigeria, especially in places like Ogoni land in Rivers State and some other oil producing states. It has severely impacted on the economic and health status of people in that region. It is important to find ways to ameliorate the harmful effects of crude oil contamination on both terrestrial and aquatic lives. In this study, we investigated the bioremediation profile of sandy and silty clay soils contaminated with crude oil. Contaminated soils were treated with inorganic fertilizer (NPK 15:15:15) and urea to improve the density of soil microbes and enzymes. The total petroleum hydrocarbon (TPH), total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB), total heterotrophic fungi (THF), total hydrocarbon utilizing fungi (THUF), and the activities of soil enzymes such as laccase, peroxidase, lipase, and catalase were investigated. The results indicated that treatment of crude oil contaminated soils with NPK and urea increased the activities of soil enzymes and enhanced total soil microbial load that play active roles in biodegradation of petroleum products, thereby suggesting that NPK and urea treatments of soils have the potential of decreasing crude oil contamination and remediating contaminated soils. Furthermore, this study showed that NPK and urea decreased the total petroleum hydrocarbon (TPH) values of both sandy (92.5%) and silty clay (80.5%) soils. The findings from this study, suggest that NPK and urea application to contaminated soils could serve as beneficial remediation agents for quick mitigation of the hazardous effects of crude oil contamination on farmlands.

Keywords: Crude oil contamination, (Bio)remediation, Soil microbes and enzymes, NPK.

Introduction

Nigeria is one of the countries with large stores of crude oil, especially in the Southern part, and due to continuous oil drilling and exploration, there are many sites, including residential areas, farmlands, rivers, and the surrounding atmosphere that have been polluted. The pollution has affected both human and animal lives, through introduction of harmful petrochemical toxicants into the environment. The toxicants cause serious damage to various tissues and organs in the human body, terrestrial animals, and aquatic lives. Also, it affects farmlands and results in poor yield of agricultural products, and as a consequence lead to poor

economic status of the inhabitants. There are many petroleum industries that operate in the Southern part of Nigeria due to their rich oil store, and their operations, over the years, have led to the pollution of these territories and have rendered many of them uninhabitable. They have caused tremendous hardship to the communities and have affected the ecosystem adversely resulting in a loss of biodiversity (Akpofure et al., 2000). Oil spillage on farmlands usually reduces yields and destroys soil fertility until there is a proper clean-up of the farmland to restore the natural ecosystem processes and reestablish stability (Sada and Odemerho, 2000).

Different methods have been employed to remediate crude oil polluted sites, reduce the damage caused, and restore the natural processes that had been altered by the pollutants. Some of the methods include natural method, also known as natural attenuation; physical methods such as booming, skimming, washing, tilting, and sediment relocation; chemical methods such as the use of detergents, dispersants, de-emulsifiers, and surface film chemicals. Another viable method that has been applied in cleaning up polluted farmlands is bioremediation, which is a biological method. It is the use of biological agents which are capable of utilizing the toxic petrochemical agents as energy source, and in the process degrade them to non-toxic forms (USEPA, 1989).

Microorganisms and some plants have been reported as key players in the bioremediation processes. When plants are used to bioremediate a polluted soil, it is termed phytoremediation. Microorganisms such as bacteria and fungi that live ubiquitously and utilize large number of petrochemical compounds as energy source are essential agents in the bioremediation processes. They can degrade the contaminants and use them as source of energy because they are supplied with various enzymes that catalyze the breakdown of these compounds. Fungi produce extracellular enzymes directly unto the contaminants and degrade them to usable and less toxic forms which are then absorbed into the cells. In the case of bacteria, the contaminants are first absorbed into the cells before they are acted upon by the degrading enzymes. The combined action of fungi and bacteria results in a clean-up of the petrochemical toxicants and bioremediation of the polluted soils. Some of the degrading microorganisms that have been identified include *Aspergillus niger*, *Pseudomonas, and Penicillium* (Cooney, 1994). The supply of required nutrients to these microorganisms may enhance their bioremediation capacity of polluted soils. Therefore, in this study, crude oil-polluted soils were treated with NPK 15:15:15 and urea fertilizer as a way of supplying the resident microorganisms with nutrients to enhance their bioremediation capacity. Hence, the use of nutrients such as NPK and urea fertilizers on bioremediation of crude soils in the Niger-Delta region of Nigeria was investigated.

Materials and Methods

2.1. Collection of Soil Sample

Soil samples were collected from an unpolluted site in Nigeria. The soil samples were collected from a depth of 0-50 cm in a sterile polythene bag.

2.2. Determination of Physicochemical Properties

Two soil types (Sandy and Silt clay) were used for this study, and their physical and chemical properties were determined using reagents of analytical grade. The following were

the physicochemical properties determined: Available phosphorus, Total nitrogen, Total Organic Carbon (TOC), pH, bulk density, porosity, water holding capacity and electrical conductivity (EC). These properties were determined three days (0, 18, and 36 day).

2.3. Soil Temperature Measurement

A pilot hole was made in the soil using a screw driver, and the thermometer was inserted into the hole making sure it's firm and kept for several minutes until a stable temperature was achieved (Hewins et al., 2016). The temperature of the soil was taken at the site.

2.4. Soil pH Determination

The method of McLean (1982) was adopted. The soil was air-dried and 10 g was placed in a 25 mL beaker. Deionized water (10 mL) was then added to the soil sample and thoroughly mixed for 5 sec with glass rod, and the suspension was allowed to stand for 30 min. The pH was measured by inserting the electrode of calibrated pH meter into soil suspension.

2.5. Determination of Bulk Density

An empty universal bottle was weighed and filled with oven-dried soil sample up to the brim by tapping, and the weight was recorded. The volume of the bottle was determined using a burette (FAO, 1980), and the bulk density was calculated as follows:

$$\text{Soil Bulk Density (g/cm}^3\text{)} = \frac{\text{weight oven dry soil}}{\text{volume of soil}}$$

2.6. Determination of soil moisture content

Gravimetric method was used for this analysis (Reynolds, 1970). It involved the determination of mass difference between the wet soil and soil oven-dried at 105ºC. An empty crucible was weighed and the mass was recorded as M_1 (g). the wet soil sample was positioned in a crucible and weighed immediately and the mass was recorded as M_2 (g). The crucible containing the wet sample was placed in an oven and dried at 105°C until a constant weight was achieved. The sample was removed from the oven and placed in a desiccator to cool. The crucible with the oven-dried soil was weighed and the mass was recorded was M_3 (g). The formular below was used to calculate moisture content:

$$\text{Moisture Content (\%)} = \frac{M2-M3}{M3-M1} \times 100$$

Where M_1 = Mass of empty crucible (g), *M_2 = Mass of crucible and wet soil (g)*, *M_3 = Mass of crucible and oven dried soil (g)*

2.7. Water Holding Capacity of Soil

A small cup with perforated bottom was weighed and recorded. Filter paper was placed at the foot of the cup and the weight was taken. Soil sample (70 g) was then weighed into the cup on a retort stand and 250 mL measuring cylinder with funnel was inserted into it. Then, 50 mL of deionized water was added and it was allowed to drain overnight. The drained water was read and recorded (FAO, 1980). Water holding capacity of soil was calculated as follows:

$$\text{WHC} = \frac{\text{Volume of water retained}}{\text{Volume of sample}} \times 100$$

2.8. Soil Porosity

Air-dried soil was added to 500 mL measuring cylinder and 100 mL of deionized water was carefully and slowly added to the soil sample inside the measuring cylinder until it reached the top of the soil. The volume of water used was recorded (FAO, 1980), and porosity was calculated as follows:

$$\text{Porosity} = \frac{\text{Amount of water added to soil sample}}{\text{Total Soil Sample}} \times 100$$

(Porosity (%) = 100- (bulk density/particle density) x 100)

2.9. Determination of Total Nitrogen in Soil

Soil sample (2 g) was weighed into an 800 mL Kjeldahl digestion flask, deionized water of 10 mL was added, followed by addition of 5 g of Kjeldahl catalyst mixture and 15 mL of 18.6 M sulphuric acid. The flask was cautiously heated on digestion stand until frothing stopped. The heat was increased until digest was clear, the flask was cooled and deionized water was slowly added and shaken. Three chips of granulated zinc were added and 30 mL of 2% boric acid were measured into 250 mL conical flask and placed under a condenser. Then, 75 mL of 10 M NaOH was slowly added into the digest in Kjeldahl flask and immediately connected to distillation apparatus. The distillate with NH_3 liberated was titrated with a standard acid along with four drops of mixed indicator (FAO, 1980). Total nitrogen was calculated as follows:

$$\text{Total Nitrogen (\%N)} = \frac{(\text{Equivalents of acid added to sample} - \text{equivalents of acid added to blank})(14.01)(100)}{\text{Sample weight (g)}}$$

2.10. Available Phosphorus in Soil

Air-dried soil (2.5 g) was placed in a clean and dried 125 mL polyethylene bottle, followed by the addition of 50 mL of 0.5 M sodium bicarbonate (pH 8.0) with polyacrylamide, which was used as the extracting solution from a dispenser. The bottle was placed in a reciprocating shaker and shook for 30 min and filtered into test tube with filter paper. Technicon II Autoanalyzer was used to determine the available phosphorus in clear filtrate by colorimeter. Soil phosphorus was calculated as:

$$\text{Soil Phosphorus (mg/kg)} = \frac{A \times B \times C \times M}{E}$$

Where, A = Sample extract reading (mg l-1), B = Extract volume (ml), C = Dilution, if performed, M = Moisture correction factor, E = Sample weight (g)

2.11. Total Petroleum Hydrocarbon (TPH)

Sandy and silty clay soil samples (5 g) contaminated with 5000 ppm of crude oil or co-treated with NPK and urea at CPN ratio of 100:2:0:2 were extracted three times with 15 mL of hexane each. The three extracts (three) were pooled and dried by solvent evaporator at room temperature under gentle nitrogen stream in a fume hood. Residues were weighed along with solvent blank beaker. The difference of the weight was calculated as the total petroleum hydrocarbon content (Chandra et al., 2013).

2.12. Gas Chromatographic Analysis of Soil

Five grams (5 g) of the soil sample was mixed with 5 g of anhydrous sodium sulphate in a 25 mL vial, 15 mL of n-hexane was added and vortexed for 10 min. The resulting soil suspension was filtered using a 0.45 µm Teflon filter. The filtrate was analyzed using a gas chromatography with flame ionization detector (GC-FID) (HP 7890). The injector and detector temperatures were programmed at 250ºC and 350ºC, respectively. The initial oven temperature was maintained at 50ºC, ramped at a rate of 5 - 280ºC per min, and held for 6 min. The injection volume for both sample and standards were 1 µL.

2.13. Soil Enzymes Assays

2.13.1. Laccase Activity

Laccase activity was determined using pyrogallol as substrate (Allison and Jastrow, 2006). Briefly, 1 g of soil sample was weighed into a 250 mL conical flask and 50 mL acetate buffer (50 mM, pH 5.0) was added. The flask was incubated at room temperature for 1 h with vigorous shaking every 20 min. The volume of the buffer was increased to 125 mL and the flask shaken vigorously. Aliquot of 10 mL of the soil suspension was transferred to a centrifuge tube and centrifuged at 4000 rpm for 10 min. The supernatant obtained was used for the enzyme assay. The test experiment contained 2 mL of the supernatant (soil suspension) and l mL substrate (25 mM pyrogallol). This was incubated in the dark at room temperature for 1 h. A sample control containing 2 mL supernatant and 1 mL buffer and also substrate control containing 1 mL substrate and 2 mL buffer were treated as the test experiment. The absorbance was measured at 460 nm using UV – Visible spectrophotometer. The buffer solution was used as blank. Laccase activity was calculated as follows:

$$\text{Laccase activity (µmol/h/g)} = \frac{\text{A} \times \text{V1}}{\text{E} \times \text{V2} \times \text{T} \times \text{W}}$$

Where, A = Net absorbance = Test – sample control – substrate control, V_1 = Volume of buffer used, E = Molar extinction coefficient for pyrogallol = 4.2/µmol, V_2 = Volume of soil suspension, T = Substrate incubation time, W = Mass of soil sample

2.13.2. Peroxidase Activity

Peroxidase activity was estimated using pyrogallol as substrate (Allison and Jastrow, 2006). The procedure described for laccase activity was followed, but with the addition of 0.2 mL of 0.3% hydrogen peroxide to the test, sample control and substrate control experiments respectively.

2.13.3. Lipase Activity

Lipase activity was determined using the method described by Parry et al (1966). Briefly, 1 g of soil sample was weighed into a 250 mL conical flask and 100 mL phosphate buffer (50 mM, pH 7.4) was added. The conical flask was shaken vigorously and then incubated at room temperature for 1 h with intermittent shaking. Thereafter, 10 mL of the soil suspension was transferred to a centrifuge tube and centrifuged at 4000 rpm for 10 min. The supernatant was used for the lipase activity assay. The test experiment was made up of 1 mL supernatant and 5 mL 10% olive oil and gum Arabic emulsion in a conical flask. The flask was incubated for 1 h on a shaker with the speed adjusted to high and temperature set at 40ºC. At the end of the incubation, 1 mL 95% ethanol was added to the conical flask and shaken to stop the reaction. The free fatty acid released was determined using a UV spectrophotometer. Control experiment was also one with 1 mL buffer solution.

Lipase activity was calculated as follows:

$$\text{Lipase activity (U/g)} = \frac{(VS-VB)\times N \times 1000}{S}$$

Where V_B = Volume of NaOH used against control flask, Vs = volume of NaOH used against experimental flask, N = Normality of NaOH, S = Volume of enzyme extract,

2.13.4. Catalase Activity

Catalase activity was determined according to the method of Cohen et al (1970). Briefly, soil sample supernatant was obtained as described for lipase activity. The supernatant (1 mL) was transferred to a test tube and 5 mL of 0.3% hydrogen peroxide was added. The content of the test tube was mixed by shaking and was then incubated at room temperature for 5 min. The reaction was stopped by the addition of 1 mL of 6 N sulphuric acid and 0.01 M $KMnO_4$ was added within 3 sec. The absorbance was measured using a spectrophotometer at 480 nm within 30-60 sec. Control experiment was performed using deionized water. Catalase activity was calculated as follows:

$$\text{Catalase activity (U/g)} = \frac{\left(\frac{\text{Absorbance}}{t}\right)\times V \times 1000}{M \times v \times W}$$

Where, Absorbance = absorbance (control) – absorbance (test), V = total volume of reaction mixture, M = molar extinction coefficient = 40.0, v = volume of supernatant used, W = mass of soil sample, t = reaction incubation time = 5 min.

2.14. Effect of Abiotic Treatment on Crude Oil Biodegradation in Soil

The ex-situ remediation method of Saterbak et al (2000) was adopted with modification. Briefly, each soil sample (3 kg) contaminated with crude oil (3000, 5000, and 8000 mg/kg) was weighed into 7 plastic buckets for each soil type (sandy soil and silty clay). Moisture content (MC) was adjusted to 60-80% of water holding capacity (WHC) for all group. Each container was agitated every three days and same with moisture adjustment. The experiment lasted for 36 days with the following determinations done every 6 days: Microbial growth were analyzed using, THB, THUB, THF, and THUF as markers.

2.15. Statistical Analysis

Experimental data were analyzed using IBM SPSS statistics 23 software for Windows. All data are expressed as mean ± SEM (standard error of mean). ANOVA was used in comparing the mean followed by Duncan's Multiple Range (DMRT), Post Hoc test. Student's t test was used to compare means when only two means were involved. Statistical significance was taken as $p < 0.05$. Correlation analysis was done to establish relationship between TPH and the other parameters evaluated (THUF, THF, THB, THUB, laccase, peroxidase, catalase, lipase activities) respectively.

Results

3.1. Physicochemical Properties of Silty Clay and Sandy Soils Contaminated with Crude Oil

The results below show the physical and chemical properties of crude oil-contaminated sandy soil and silt clay.

Table 1. *Physicochemical data on silty clay and sandy soils contaminated with crude oil. Values are expressed as mean ± SEM (n = 3, at 0, 18, and 36 day).*

Parameters	Silty clay contaminated				Sandy soil contaminated			
	Control	**3000 ppm**	**5000 ppm**	**8000 ppm**	**Control**	**3000 ppm**	**5000 ppm**	**8000 ppm**
pH	6.84 ± 0.05	5.70± 0.01	5.50± 0.04	5.70± 0.03	6.33 ± 0.31	6.71±0.13	6.51± 0.36	6.80± 0.63
Total nitrogen (mg/kg)	0.49 ± 0.00	0.18± 0.03	0.22± 0.01	0.27± 0.49	0.43 ± 0.76	0.17±0.51	0.21± 0.11	0.22± 0.11
Available phosphorus (mg/kg)	7.10 ± 2.38	4.42± 1.10	4.48± 1.15	4.53± 2.20	6.10 ± 2.09	3.42±1.25	3.48± 1.32	3.53± 1.10
TOC (%)	0.07 ± 0.4	0.104 ±0.2	0.21± 0.21	0.43± 0.12	0.06 ± 0.16	0.10±0.12	0.29± 0.01	0.43± 0.19
Potassium	8.91 ± 2.01	6.55± 1.98	6.59± 1.52	6.61± 1.31	6.93 ± 1.65	4.54±1.11	4.57± 1.21	4.59± 1.41
TPH (mg/kg)	<1	7568	7601	7608	<1	7568	7601	7608
Moisture content (%)	0.30± 0.02	0.32± 0.04	0.74± 0.01	1.32± 0.05	0.25± 0.08	0.28±0.01	0.69± 0.03	1.27± 0.06
Soil bulk density (g/cm^3)	1.09± 0.02	2.87± 0.12	2.92± 0.15	2.02± 0.98	1.69± 0.76	2.81±0.23	3.94± 0.31	4.08± 0.68
Conductivity (µs/cm)	180± 3.10	190± 2.87	170± 1.99	190± 2.55	160± 2.20	170± 2.01	150± 1.89	170± 1.77
Porosity	0.51± 0.04	0.36± 0.07	0.31± 0.04	0.29± 0.07	0.31± 0.02	0.33±0.01	0.36± 0.09	0.39± 0.07
Water holding capacity	0.19± 0.00	0.19± 0.01	0.19± 0.01	0.19± 0.01	0.16 ± 0.03	0.16±0.02	0.16± 0.03	0.16± 0.02
Temperature ºC	27							
Particle size distribution								
* Silty clay %	72.1							
* Sand %	93							

3.2. Physicochemical Properties of Contaminated Silty Clay and Sandy Soils Treated with NPK and Urea Fertilizers.

The results below show the physical and chemical properties of contaminated sandy soil and silty clay treated with NPK 15:15:15 and urea at a CNP (carbon, nitrogen, and phosphorus) ratio of 100:2:0:2.

Table 2. *Impact of NPK and urea fertilizers on the physicochemical properties of silty clay and sandy soils contaminated with crude oil. Values are expressed as mean ± SEM (n = 3, at 0, 18, and 36 day).*

Parameters	**Treated silty clay**			**Treated sandy soil**		
	3000 ppm	**5000 ppm**	**8000 ppm**	**3000 ppm**	**5000 ppm**	**8000 ppm**
pH	5.69±0.31	6.18±0.33	6.44±0.10	8.36±0.21	8.28±0.19	8.44±0.23
Total Nitrogen (mg/kg)	0.31±0.01	0.37±0.04	0.42±0.02	0.28±0.11	0.31±0.12	0.39±0.22
Available Phosphorus (mg/kg)	6.19±1.09	6.38±1.23	6.77±1.32	5.14±0.98	5.33±0.87	5.74±0.92
TOC %	1.26±0.21	2.02±0.12	4.48±0.31	1.26±0.23	2.02±0.22	4.48±0.31
Potassium	72.4±4.01	73.5±3.81	74.8±3.17	70.6±2.12	71.4±2.02	72.1±1.87
THC (mg/kg)	634±3.02	619±3.41	540±3.44	634±3.11	619±2.45	540±3.65
Moisture content (%)	3.81±0.33	3.93±0.23	4.02±0.31	3.76±0.43	3.88±0.14	3.97±0.22
Soil bulk density (g/cm^3)	3.17±0.12	3.28±0.22	3.39±0.12	3.72±0.19	3.86±0.21	3.91±0.32
Conductivity (µs/cm)	230±4.40	200±3.98	228±3.99	210±3.01	180±3.41	208±3.06
Porosity	0.24±0.01	0.34±0.02	0.35±0.01	0.42±0.04	0.44±0.02	0.43±0.01
Water Holding Capacity	1.24±0.15	1.24±0.57	1.26±0.43	1.22±0.34	1.22±0.32	1.24±0.48
Temperature ^oC	26			27		
Particle Size Distribution *Silty clay (%): 72.1 *Sandy soil (%): 93						

3.3. Effect of NPK and Urea on Soil Enzymes Activity

To investigate the effect of NPK and urea fertilizer treatment on enzymes activities of contaminated soils. Two different soil types, silty clay and sandy, contaminated with crude oil, were treated with NPK and urea fertilizers. The results indicated that the activities of soil enzymes were affected by applied treatment during the 36 days period. An increased activity was observed for laccase from days 0-18 on both soil types across the treated groups. However, laccase activity in sandy soil was higher compared to that of silty clay (Fig. 1.A). Peroxidase activity increased in 12 days from the start of the experiment across treated groups in both soils. Peroxidase activity in silty clay was higher than that of sandy soil (Fig. 1.B). Furthermore, an increased activity was recorded for catalase in sandy soil and silty clay with the highest peak at day 18. Catalase activity in sandy soil was higher than that of silty clay (Fig. 1.C). Also, lipase activity increased from days 0-12 in sandy soil while for silky clay, the highest activity was on day 24 (Fig. 1.D). Taken together, these results suggested that treatment of crude oil contaminated soils with NPK and urea fertilizers has the potential of enhancing soil enzymes activity that function in degrading crude oil contaminant.

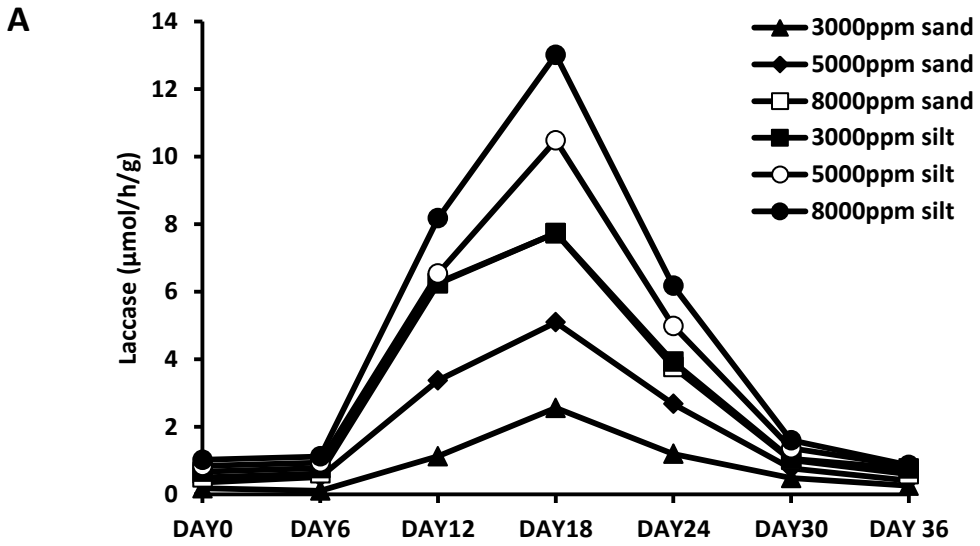


Fig. 1. (A) *Effect of NPK and urea fertilizers on soil enzymes activities*. Effect of NPK and urea on soil laccase activity. *Values are expressed as mean ± SEM (n = 3). P < 0.05.*

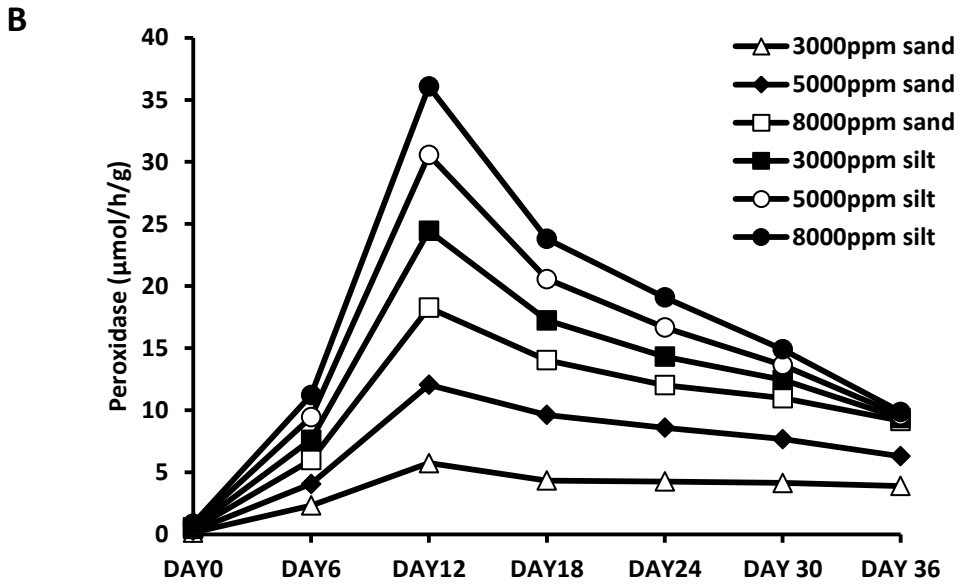


Fig. 1. (B) *Effect of NPK and urea fertilizers on soil enzymes activities - Effect of NPK and urea on soil peroxidase activity. Values are expressed as mean ± SEM (n = 3). P < 0.05.*

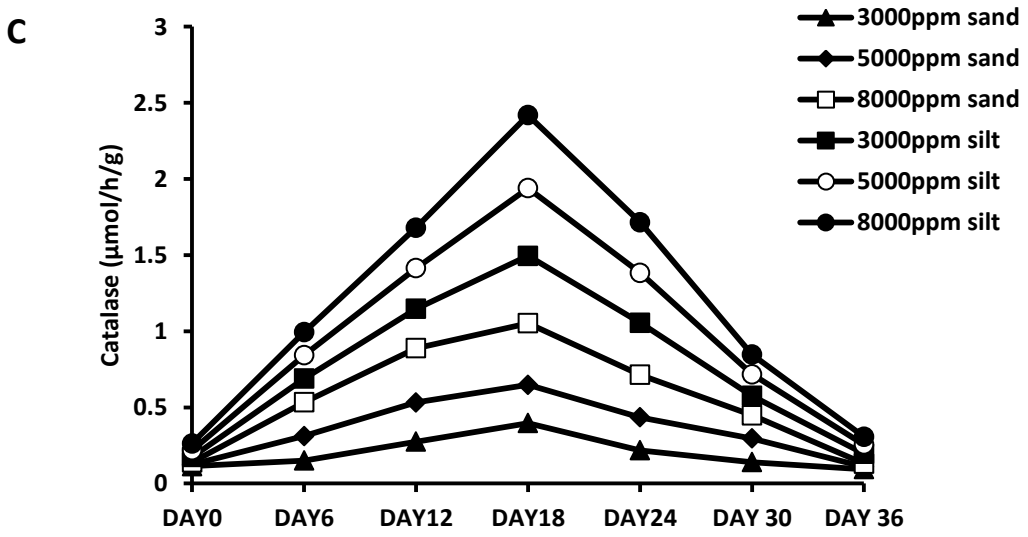


Fig. 1. (C) *Effect of NPK and urea fertilizers on soil enzymes activities. Effect of NPK and urea on soil catalase activity. Values are expressed as mean ± SEM (n = 3). P < 0.05.*

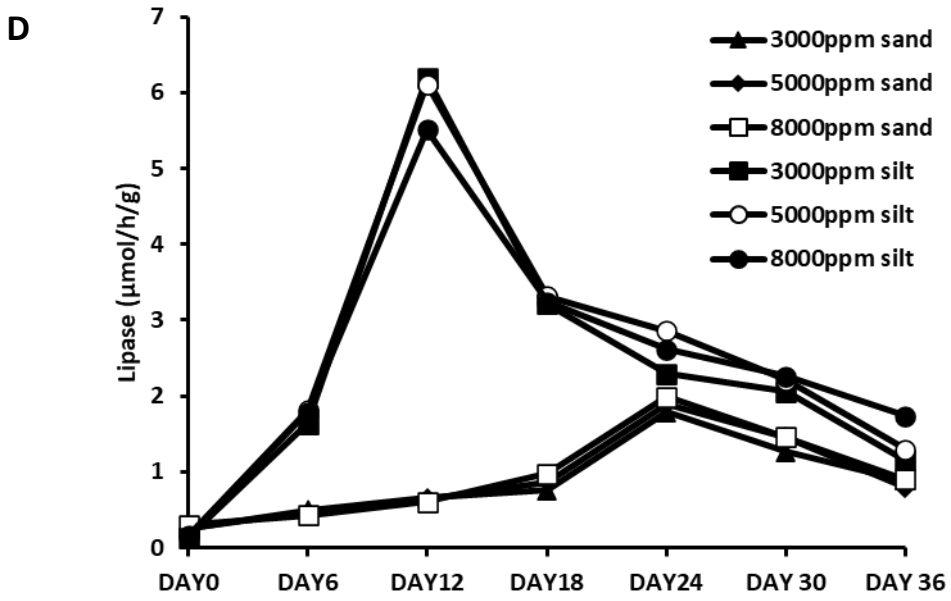


Fig. 1. (D) *Effect of NPK and urea fertilizers on soil enzymes activities. Effect of NPK and urea on soil lipase activity. Values are expressed as mean ± SEM (n = 3). P < 0.05.*

3.4. Effect of NPK and Urea Treatment on Soil Microbial Load

To investigate the effect of NPK and urea fertilizers on soil microbial load. Sandy and silty clay soils were treated with NPK 15:15:15 and urea, and the microbial load was monitored and reported as CFU/g during the 36 days period of treatment. An increase in amount of total hydrocarbon utilizing fungi (THUF) and total heterotrophic fungi (THF) were observed from day 0 and peaked on day 12 for both sandy and silty clay soils before decreasing thereafter in all the treatments (Fig. 2.A and B). The same pattern of growth was observed for total hydrocarbon utilizing fungi (THUB) and total heterotrophic fungi (THB) with maximum peak on day 18 (Fig. 2.C and D). These results indicated that treatment of crude oil contaminated soils with NPK and urea fertilizers enhanced the total soil microbial load. Increased soil microbial load results in increased degradation of crude oil contaminant in the soils and hence helps to ameliorate the toxic effects of crude oil on soils.

A

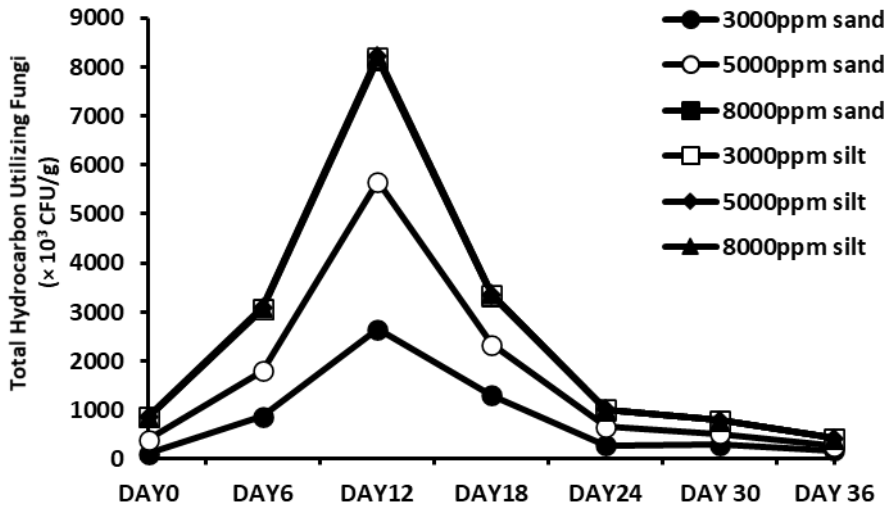


Fig. 2. **(A)** *Effect of NPK and urea on soil microbial load of soil contaminated with crude oil. Effect of NPK and urea on THUF of sandy and silty clay soils. Values are expressed as mean ± SEM (n = 3). P < 0.05.*

B

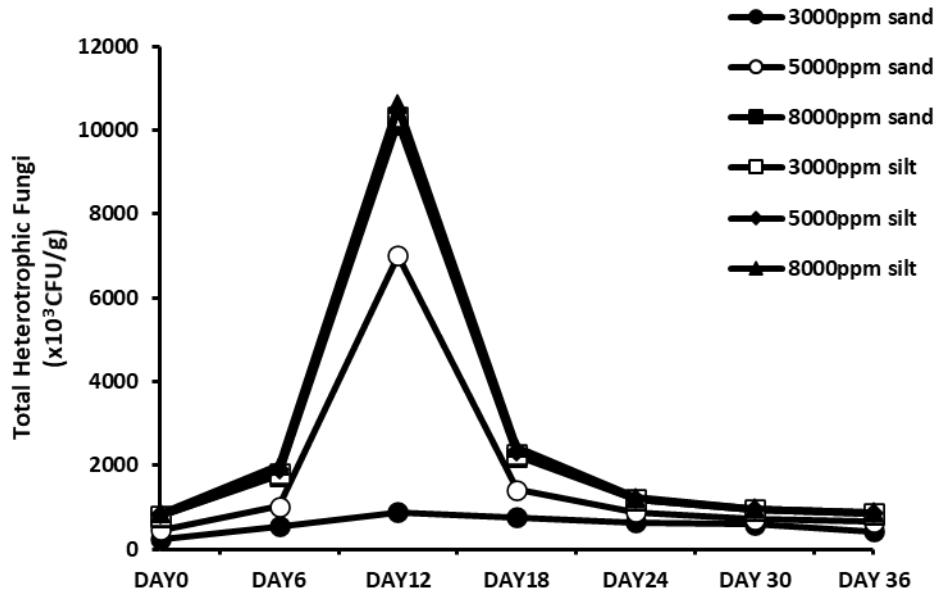


Fig. 2. *(B) Effect of NPK and urea on soil microbial load of soil contaminated with crude oil. Effect of NPK and urea on THF of sandy and silty clay soils Values are expressed as mean ± SEM (n = 3). P < 0.05.*

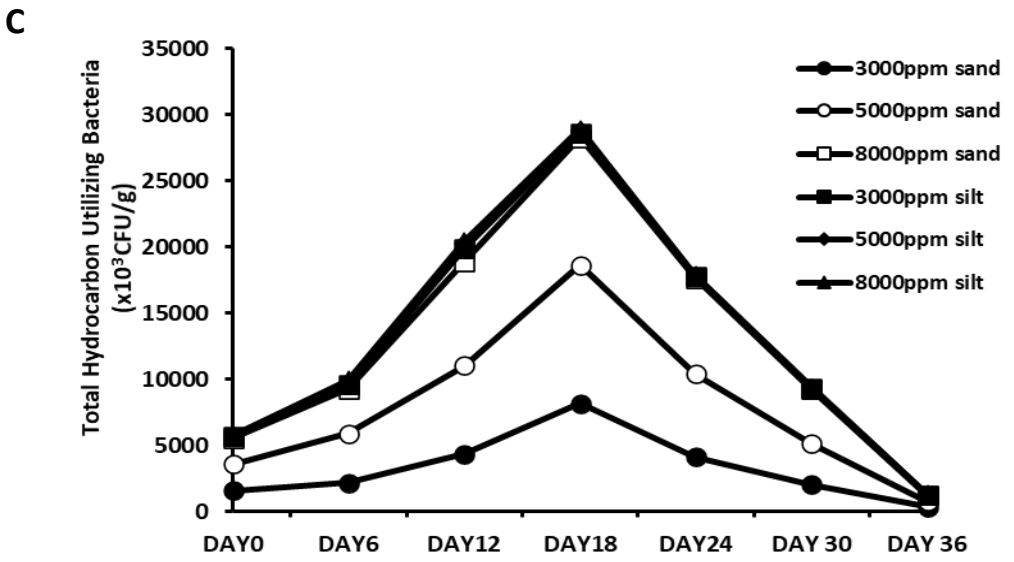


Fig. 2. (C) *Effect of NPK and urea on soil microbial load of soil contaminated with crude oil. Effect of NPK and urea on THUB of sandy and silty clay soils. Values are expressed as mean ± SEM (n = 3). P < 0.05.*

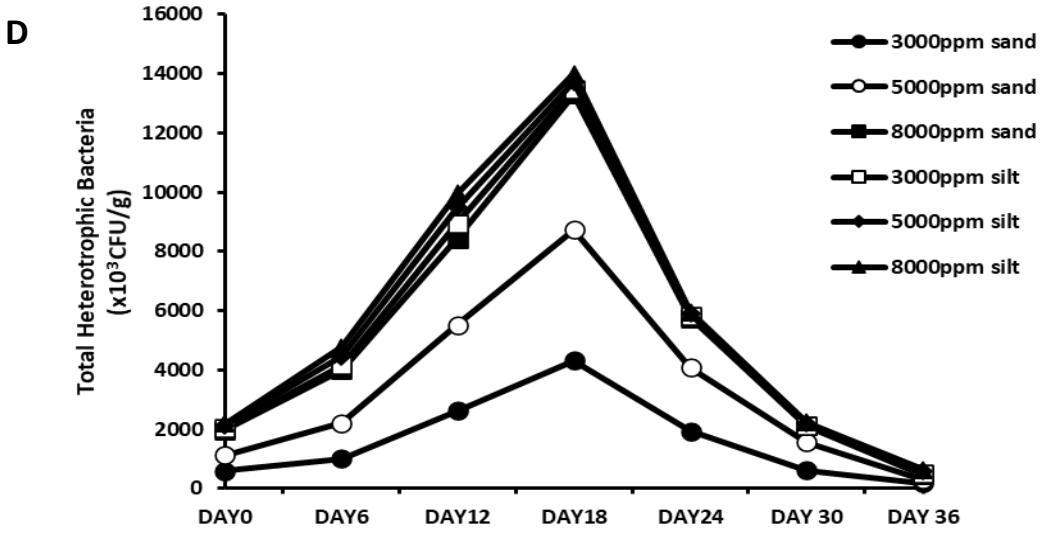


Fig. 2. (D) *Effect of NPK and urea on soil microbial load of soil contaminated with crude oil. Effect of NPK and urea on THB of sandy and silty clay soils. Values are expressed as mean ± SEM (n = 3). P < 0.05.*

3.5. Effect of NPK and Urea Treatment on Total Petroleum Hydrocarbon

The effect of NPK and urea fertilizers on oil degradation at 5000 ppm on sandy soil and silty clay was investigated. A significant decrease in total petroleum hydrocarbon (TPH) concentration was observed in both soil types. Comparatively, the rate of TPH reduction in sandy soil was higher than that of silty clay as the former having 92.90% TPH reduction and the latter having 80.46% after the 36 days enhanced treatment.

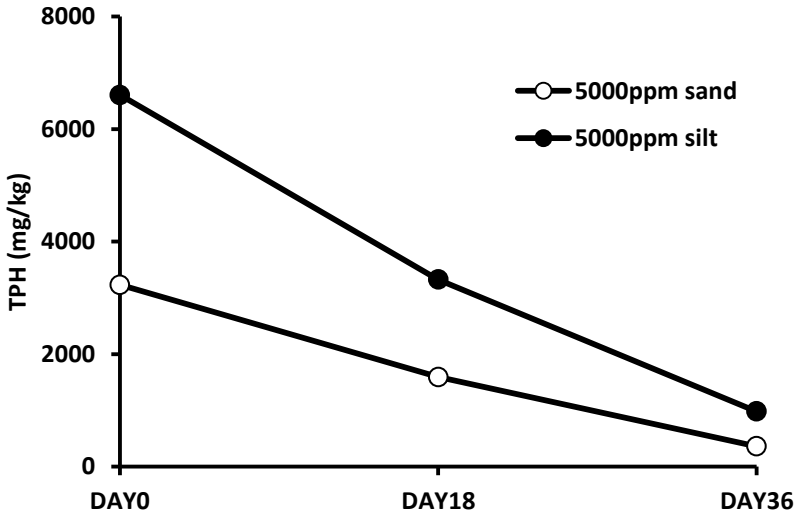


Fig. 3. *Effect of NPK and urea on TPH of soils contaminated with crude oil. Values are expressed as mean ± SEM (n = 3). P < 0.05.*

Discussion

Petrochemical discharge from crude oil industries on farmlands is one of the major problems faced by some parts of Nigeria. These petroleum products are harmful to body tissues and organs resulting in life-threatening disease conditions. Plants and aquatic lives are affected as well by the toxic nature of these substances. Therefore, there is an urgent need to clean up the pollution to restore normalcy of the ecosystem. This study investigated the additional effects of nutrients, in the form of NPK and urea fertilizers, on the bioremediation of crude oil-polluted soils. The results indicated that crude oil polluted soils had higher electrical conductivity, bulk density, and water holding capacity as compared to the unpolluted soil. This was in agreement with the report of other investigators (Barua et. al., 2011). However, other properties as pH, total organic carbon, potassium, moisture content, and phosphorus were higher in the uncontaminated soil compared to the contaminated sample (Table 1 and 2). Crude oil in the soil is known to affect the properties and productivity of soil (Bosma et al., 1997; Michel and Fingas, 2016). The sandy nature, porosity and the pH of the contaminated soil supports leaching and possible contamination of the ground water (Bosma et al., 1997; Michel and Fingas, 2016). Inorganic nutrient sources like NPK 15:15:15 and urea

with nitrogen content of 46.6% was used in this study, because the quantification of required nutrient level in bioremediation studies is easier when inorganic nutrients are used (Suja et al., 2014; Shahi and Aydin, 2016).

Laccase and peroxidase are key extracellular oxidative enzymes produced by oleophilic microbes (Bach et al., 2013; Godoy et al., 2016). They were assayed in this study and a negative relationship was established between them (oxidative enzymes activity) and TPH using Pearson correlation. This indicated that increase in oxidative enzyme activity corresponded with decrease in TPH in soils (Fig. 1.A and B). However, the correlation between enzyme activity and microbial load was significant and positive indicating a corresponding increase of both enzyme activity and microbial load during the degradation process in both sandy soil and silt clay. The activities of catalase and lipase were used to monitor the reduction in toxicity level following oil degradation in soil (Margesin and Zimmerbauer, 1999; Riffaldi et al., 2006; Mahmoud et al., 2016), because they provide useful information about the soil quality. There was an increase in activity of both catalase and lipase in all treated variants. Catalase activity in soil decreased with increasing oil concentration (Fig. 1c). Wu et al (2016) corroborated this finding and affirmed the suitability of catalase activity for monitoring the bioremediation of crude oil. Other researchers including Achuba and Peretiemo-Carke (2008), Ajao et al (2011), and Achuba and Okoh (2014) have all reported increase in catalase activity during bioremediation as a mark of increased microbial activity. Similar result was observed in this study when catalase activity was monitored in treated sandy soil and silty clay. Lipase catalyzes the hydrolysis of glycerides at water-lipid interface to enable the hydrolysis products of lipids to be absorbed and used for metabolic activities by the microbes (Margesin and Zimmerbauer, 1999; Mahmoud et al., 2016). It was used by Margesin and Schinner (2001) to monitor the degradation of hydrocarbon in the soil. This study showed a decrease in lipase activity of contaminated soil (Fig. 1.C and D), but the activity increased in the treated variants. This result is in agreement with the report of other researchers who also reported a negative correlation of lipase activity and reduction in TPH (Margesin and Schinner, 2001). Achieving the desired CNP ratio is an important consideration in bioremediation optimization (Bach et al., 2013). Microbial activity could be inhibited when limiting nutrients are supplied at high concentration to the bioremediation system, hence it is important to measure the optimum CNP ratio (Huesemann, 1994). The CNP ratio of 100:10:1 has been reported to be the optimum ratio for oil bioremediation in soil (Huesemann, 1994; Wu et al., 2016). In this study, we recorded 92.95 ± 0.02% for sandy soil and 80.46 ± 0.0311% for silty clay degradation with CNP ratio of 100:2:0.2 after 36 days (Fig. 2). This indicated that there was a high reduction in TPH for both soils. The treatments increased soil microbial load monitored by THB, THUB, THF, THUF analysis (Fig. 3). Increase in the bacterial load was rapid from days 0-18 like the increase in fungal load from days 0-12.

The bioremediation was successful in both sandy and silty clay soils with 93% and 80.5% degradation of TPH respectively (Fig. 3). However, the reason for the lower TPH degradation rate in silty clay soil than sandy soil could be due to inefficient oxygen transfer in the soil. Fine grained clay with high surface area formed a sticky texture in the presence of water, blocking efficient oxygen transfer through the soil. Sandy soil on the other hand is more porous than silty clay. Higher porosity allows better oxygen transfer in the soil which is

essential to biodegradation of hydrocarbon. Larger pores also provide enough space for microbial growth. It has been demonstrated that pores smaller than 3 μm are not accessible to bacteria (Aislabie and Deslippe, 2013).

Another reason for low degradation of hydrocarbon in silty clay could be strong adsorption of the pollutant on the surface of soil particles. For the treated sandy soil, the crude oil residue on the 36th day of bioremediation was 362.5 mg/kg while that of silty clay was 418.5 mg/kg on day 36, indicating that oil remediation in sandy soil was faster compared to silt clay.

Conclusion

Crude oil pollution of soil has become recurrent in Nigeria with little or no solution due to over dependence on oil as the main source of revenue for the country. Therefore, considering the negative impact on the ecosystem, bioremediation techniques which include the use of fertilizers such as NPK and urea, in amounts that will not further compound the environmental and climate change problems, is highly recommended.

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