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ORIGINAL ARTICLE

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Impact of a combination of NPK and urea fertilizers on bioremediation of soil contaminated with crude oil

May Chidinma Ilonzeh

Department of Biochemistry University of Benin, Edo State, Nigeria

ABSTRACT

This research, centered on the impact of a combination of inorganic fertilizers (NPK and Urea) on bioremediation as an alternative of the use of solely one fertilizer. The effect of bio-stimulation on crude oil degradation in contaminated soil was investigated in six therapy group and C:N:P ratio of 100:2:0.2 were used. The microbial growths were measured as total heterotrophic bacteria, total hydrocarbon utilizing bacteria, total heterotrophic fungi and total hydrocarbon utilizing fungi. Laccase, Peroxidase, Lipase and Catalase activities as well as residual petroleum hydrocarbon were assayed every 6 days for 36 day. From this study, the outcomes acquired from the chemical analyses published that water holding capacity, moisture content, bulk density were higher in the treated soil; while organic carbon, electrical conductivity were higher in contaminated soil and porosity, whole nitrogen and complete accessible phosphorus are higher in uncontaminated soil. The study also shows that treatment with the inorganic fertilizer increased the activities of soil enzymes, soil microbial load and decreased in total petroleum hydrocarbon. The effects acquired from this findings suggests that combination of inorganic fertilizers and C:N:P ratio adopted shortened the time frame for bioremediation of crude oil in opposition to when only NPK is been applied at 10% as suggested via other researchers.

KEYWORDS

NPK fertilizer; Urea fertilizer; Bioremediation; Soil microbe's; Soil enzymes; Crude oil contamination

ARTICLE HISTORY

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Introduction

Crude oil is a fossil fuel, a natural aid from decaying plant life and animals over thousands of hundreds of years ago (in most locations; crude oil can be positioned alongside sea beds). Crude oil diffs in color, from clear to tar-black and in viscosity, from water to almost stable. This is as a stop end result of its relative abundance, excessive power density and handy transportability to extraordinary areas. Basically, crude oil is quint essential to industries and it accounts for a large percentage world's energy consumption [1]. The world at titanic consumes 30 billion barrels (4.8km^3) of oil per 12 months and pinnacle oil buyers in the main consist of developed nations. This create it one of the world's most necessary commodities [2].

Supplementally, crude oil act as uncooked fabric for many chemical products, together with fertilizers, pharmaceuticals, plastics, pesticides and solvents [3]. It is also an electricity supply powering the significant majority of vehicles. Oil consists of hydrocarbons, which encompass aromatic hydrocarbons, cycloalkanes and alkanes while other natural compounds incorporate nitrogen, oxygen and sulphur. Crude oil additionally incorporates organo-metallic complexes containing nickel and vanadium in precise deal smaller proportions compared to the specific constituents. In spite of these organo-metallic compounds are troublesome throughout crude oil refining. Invariably, oil spillage damages the soil, water, every plant and animals lifestyles. Consequent upon its contents of lead, oil pollution renders soils unproductive for years after spillage, decreasing the growth ordinary performance of flowers [4]. Therefore, plant boom and establishment, and re-vegetation of polluted area can serve as warning signs for soil recovery [5]. Numerous techniques have been employed to get rid of oil wastes and its derivatives from soil and water. These encompass bodily (spray, vapor extraction, stabilization, solidification), chemical (photo-oxidation, dissolution, detergent use), and biological strategies (bioremediation). All these techniques can be employing in the remedy of contaminated region relying on the priorities and situations of each case.

In contrast to these methods, addition of sufficient concentration of vitamins to swimsuit the nature and extent of hydrocarbon modern in the soil is of high importance. These nutrient even though exist in contaminated soils but inadequate for the remediation gadget. For that purpose, informing the need to observe additional nutrient in structure of fertilizers to optimize the necessary limiting nutrient, Nitrogen (N) and Phosphorus (P). These nutrient steadiness's out the multiplied concentrations of Carbon in the polluted areas prompted through potential of the pollution thereby helping microbial metabolism in areas of the biosynthesis of amino acids, proteins and nucleic acids that aid to shape a proper microbial biomass and high levels of metabolic activity. However excessive nutrient attention can inhibit the biodegradation process.

***Correspondence:** May Chidinma Ilonzeh, Department of Biochemistry University of Benin, Edo State, Nigeria, e-mail: mayilonzeh@gmail.com

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Effective remediation has been documented to require Carbon, Nitrogen, Phosphorus ratios(C: N: P) between 100:10:1, 100:1:0.5 and 100:2:0.2, 100:1:0.1. Though according to their documentary, the above ratios are excess because all the carbon current in the soil are no longer for biomass formation and there is regular recycling of nitrogen and phosphorus by using microorganisms. 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.

Materials and Methods

Collection and preparation of soil samples

Collection of soil sample:

The unpolluted soil specimen was obtained from the Department of Soil Science, University of Benin, Nigeria.

Soil sample preparation and contamination:

Five kilogram of unpolluted soil was weighed into seven plastic containers which were air dried for five days; sieved using 2mm sieve to remove plant material and large particles. Then, 5g of soil was weighted into six plastic buckets of which it was chemically polluted with crude oil. Crude oil (obtained from shell) was applied to the six bucket at various ppm (3000 ppm, 5000 ppm, and 8000 ppm) in a way that would homogeneously pollute the whole surface. The drug, which is a complex mix of various quantities of inorganic fertilizers (urea and NPK), was then dispersed in three plastic containers correlating to those infected. Three times a week the various soil containers were agitated to made available the required ventilation and mixing of nutrients and microbes with the contaminated soil [6].

Determination of physicochemical properties:

Sandy loan soil was 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).

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 [7]. The temperature of the soil was taken at the site.

Soil pH determination:

The soil was air-dried and 10g 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 [8].

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 [9], and the bulk density was calculated as follows:

Soil Bulk Density (g/cm^3) = (weight oven dry soil)/(Volume of soil)

Determination of soil moisture content:

Gravimetric method was used for this analysis [10]. It involved the determination of mass difference between the wet soil and soil oven-dried at 105C. 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 formula below was used to calculate moisture content:

$$\text{Moisture Content (\%)} = (M_2-M_3 \times 100)/(M_3-M_1)$$

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).

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 (70g) 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 [9].

Water holding capacity of soil was calculated as follows:

WHC = (Volume of water retained × 100)/(Volume of sample)

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 [9], and porosity was calculated as follows:

Porosity = (Amount of water added to soil sample × 100)/(Total soil sample)

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

Determination of total nitrogen in soil:

Soil sample (2g) was weighed into an 800 ml Kjeldahl digestion flask, deionized water of 10 ml was added, followed by addition of 5g 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 [9]. Total nitrogen was calculated as follows:

Total Nitrogen (%N) =

((Equivalents of acid added to sample-equivalents of acid added to blank)(14.01)(100))/(Sample weight (g))

Available phosphorus in soil:

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Air-dried soil (2.5g) 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:

Soil Phosphorus (mg/kg) = AXBXCXM/(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)

Total petroleum hydrocarbon (TPH):

Soil samples (5g) contaminated with 5000 ppm of crude oil or 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 [11].

Gas chromatographic analysis of soil:

Five grams (5g) of the soil sample was mixed with 5g of anhydrous sodium sulphate in a 25 ml vial, 15 ml of n-hexane was added and vortexes 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 250°C and 350°C, respectively. The initial oven temperature was maintained at 50°C, ramped at a rate of 5 - 280°C per min, and held for 6 min. The injection volume for both sample and standards were 1 µl.

Soil enzymes assays

Laccase activity:

Laccase activity was determined using pyrogallol as substrate [12]. Shortly, 1g 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 1 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:

Laccase activity (µmol/h/g) = (A X V_1)/(E X V_2 X T X 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

Peroxidase activity:

Peroxidase activity was estimated using pyrogallol as substrate [12]. 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.

Lipase activity:

Lipase activity was determined using the method described by Parry et al (1966) [13]. Briefly, 1g 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 40°C. 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:

Lipase activity (U/g) = ((VS-VB) X N X1000)/(S)

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

Catalase activity:

Catalase activity was determined according to the method of [14]. 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 acids 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:

Catalase activity (U/g) = ((Absorbance /t) x v x1000)/(M x V x 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.

Effect of abiotic treatment on crude oil biodegradation in soil

The ex-situ remediation method of was adopted with modification [15]. Briefly, each soil sample (3 kg) contaminated with crude oil (3000, 5000, and 8000 mg/kg) was weighed into 7 plastic buckets for soil. Moisture content (MC) was adjusted to

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60-80% of water holding capacity (WHC) for all groups. 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 was analyzed using, THB, THUB, THF, and THUF as markers.

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 and Discussion

Physicochemical properties of sandy loam soils contaminated with crude oil

The results below shows the physical and chemical properties of crude oil contaminated sandy loam soil.

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

Parameter	Control	3000 ppm	5000 ppm	8000 ppm
pH	6.5±0.31	6.7±0.33	6.7±0.33	6.5±0.10
Total Nitrogen (mg/kg)	0.034±0.13	0.021±0.01	0.028±0.06	0.024±0.04
Available Phosphorus (mg/kg)	2.81±0.96	2.27±0.87	2.42±0.76	2.16±0.78
TOC (%)	0.14±0.06	0.66±0.23	0.78±0.28	0.78± 0.28
Potassium	16.30±0.22	14.80±0.20	29.11±0.28	11.40±0.03
TPH (mg/kg)	< 1	7568	7601	7608
Moisture Content (%)	9.83±0.06	9.31±0.36	10.98±0.32	10.47±0.30
Soil Bulk Density (g/cm3)	2.42±0.12	2.84±0.15	2.86±0.18	3.24±0.31
Conductivity (µs/cm)	220±4.40	210±3.98	140±2.87	200±2.99
Porosity	0.48±0.004	0.34±0.02	0.36±0.01	0.34±0.02
Water Holding Capacity	0.25±0.43	0.25±0.43	0.25±0.43	0.25±0.43
Sulphate mg/kg	20±0.12	12±0.03	16±0.08	12±0.03
Temperature	27 °C			
Particle Size Distribution				
Sand	74.60%			
Silt	12.00%			
Clay				

Physicochemical properties of contaminated sandy loam soils treated with inorganic fertilizers

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

Effect of NPK and urea on soil enzymes activity

To investigate the effect of NPK and urea fertilizers on soil microbial load. The results below 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 across the treated groups. Peroxidase activity increased in 12 days from the start of the experiment across treated soils. Furthermore, an increased activity was recorded for catalase with the highest peak at day 18. Also, lipase activity increased from days 0-12 with highest activity was on day 24. Summarily , these results indicates that treatment of crude oil contaminated soils with combination NPK and urea fertilizers has the potential of enhancing soil enzymes activity that function in degrading crude oil contaminant.

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

Parameter	3000 ppm	5000 ppm	8000 ppm
pH	6.8±0.21	6.8±0.21	6.5±0.10
Total Nitrogen (mg/kg)	0.029±0.12	0.030±0.24	
Available Phosphorus (mg/kg)	2.76±0.88	2.80±0.98	2.5±0.89
TOC (%)	0.48±0.02	0.52±0.08	0.60±0.06
Potassium	2±0.05	18±0.16	18±0.16
TPH (mg/kg)	634±3.11	619±2.45	540±3.65
Moisture Content (%)	12.62±0.14	14.8±0.21	14.85±0.28
Soil Bulk Density (g/cm3)	2.62±0.23	2.62±0.23	2.80± 0.31
Conductivity (µs/cm)	230±3.10	215±3.09	235±3.30
Porosity	0.42±0.04	0.42±0.04	0.38±0.01
Water Holding Capacity	0.30±0.40	0.30±0.40	0.32±0.44
Sulphate mg/kg	18±0.10	20±0.12	22±0.15
Temperature	27 °C		
Particle Size Distribution			
Sand	74.60%		
Silt	12.00%		
Clay	13.40%		

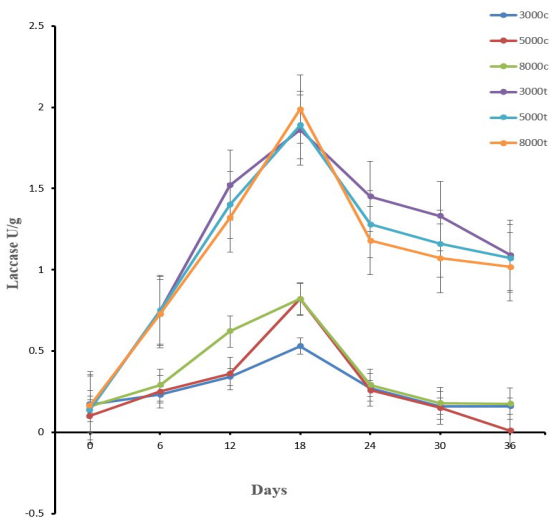

Figure 1. Effect of NPK and urea fertilizers on soil enzymes activities. Effect of NPK and urea on soil laccase activity. Values are expressed as mean ± SEM (n = 3). P < 0.05.

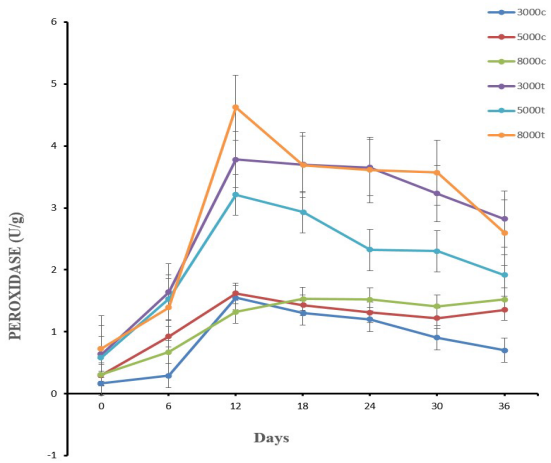

Figure 2. Effect of NPK and urea fertilizers on soil enzymes activities - Effect of NPK and urea on soil peroxidase activity. Values are expressed as mean ± SEM (n = 3). P < 0.05.

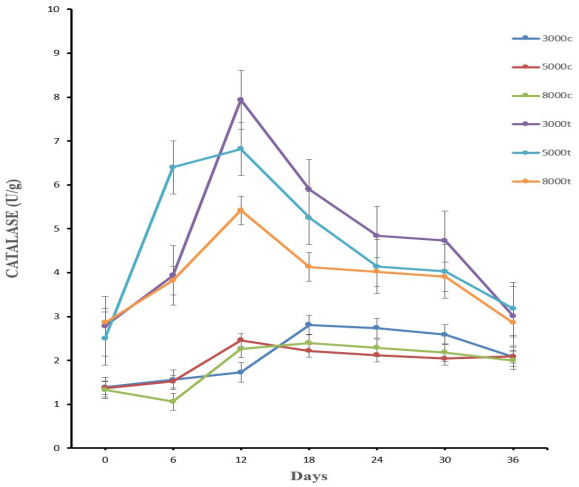

Figure 3. Effect of NPK and urea fertilizers on soil enzymes activities. Effect of NPK and urea on soil catalase activity. Values are expressed as mean ± SEM (n = 3). P < 0.05

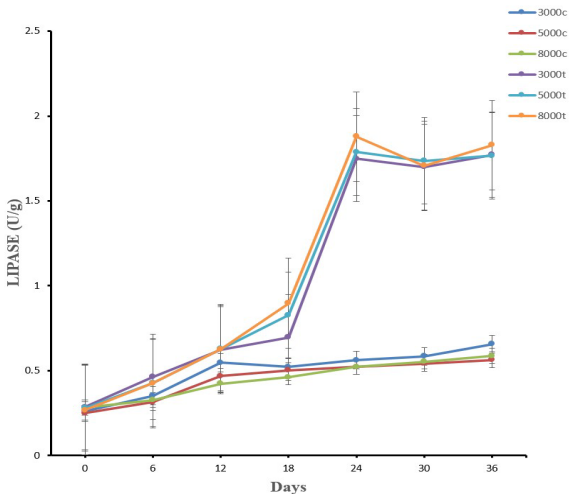

Figure 4. Effect of NPK and urea fertilizers on soil enzymes activities. Effect of NPK and urea on soil lipase activity. Values are expressed as mean ± SEM (n = 3). P < 0.05.

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Effect of NPK and urea treatment on soil microbial load

To investigate the effect of NPK and urea fertilizers on soil microbial load. The soils was 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 before decreasing thereafter in all the treatments (Figure 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 (Figure 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.

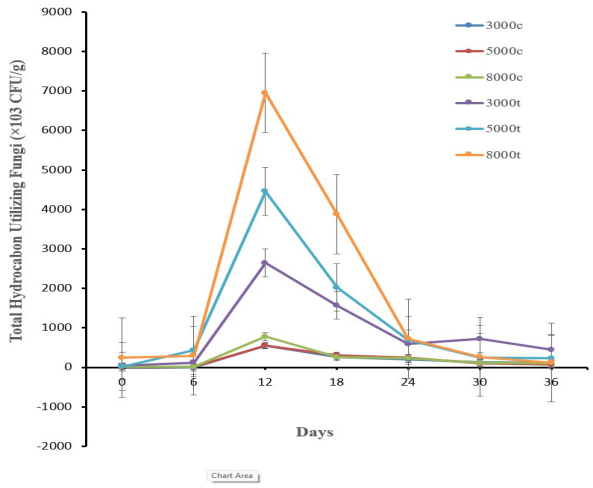

Figure 5. Effect of NPK and urea on soil microbial load of soil contaminated with crude oil. Effect of NPK and urea on THUF of sandy and silty clay soils. Values are expressed as mean ± SEM (n = 3). P < 0.05.

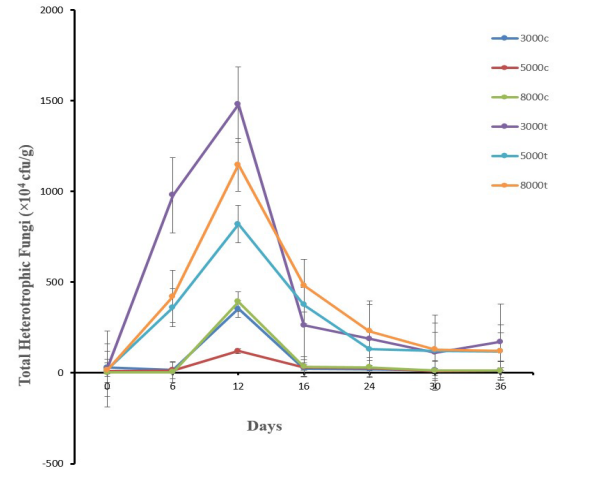

Figure 6. Effect of NPK and urea on soil microbial load of soil contaminated with crude oil. Effect of NPK and urea on THF sandy loam soils Values are expressed as mean ± SEM (n = 3). P < 0.05.

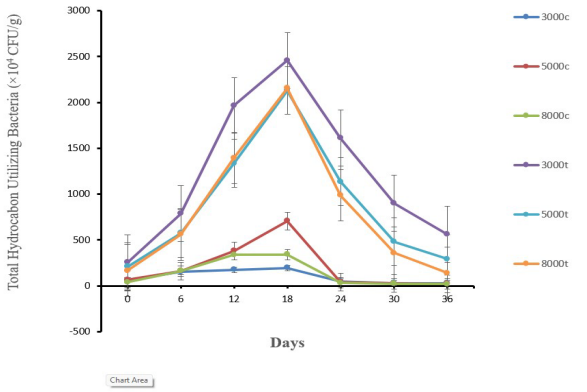

Figure 7. Effect of NPK and urea on soil microbial load of soil contaminated with crude oil. Effect of NPK and urea on THUB of sandy loam soils. Values are expressed as mean ± SEM (n = 3). P < 0.05

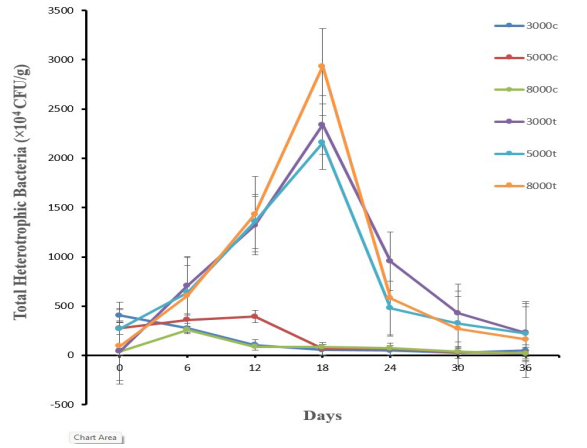

Figure 8. Effect of NPK and urea on soil microbial load of soil contaminated with crude oil. Effect of NPK and urea on THB of sandy loam soils. Values are expressed as mean ± SEM (n = 3). P < 0.05

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 loam soil was investigated. A significant decrease in total petroleum hydrocarbon (TPH) concentration was observed in sandy loam soil.

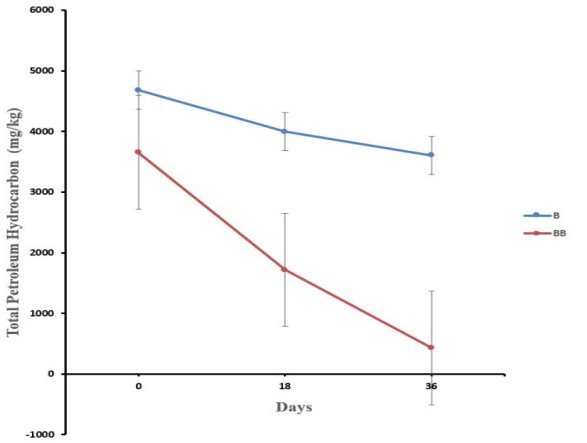

Figure 8. Effect of NPK and urea on TPH of soils contaminated with crude oil. Values are expressed as mean ± SEM (n = 3). P < 0.05

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Discussion

The bioremediation of hydrocarbon polluted soil was investigated using combination of urea and NPK fertilizers. Among all strategies to speed up the biological breakdown of hydrocarbons in soil, bio-stimulation of the intrinsic microorganisms by addition of nutrients is the most frequently used bioremediation technique as the contaminant introduces enormous amount of carbon source which tends to result in rapid depletion of the available nitrogen and phosphorus that are essential for microbial growth.

Soil microbes need nutrients like carbon, nitrogen and phosphorus to support their activities [16,17]. Crude oil pollution decreased the availability of nitrogen and phosphorus in the soil (table 1) [17]. Addition of inorganic nutrient to the contaminated soil can help to stimulate and improve microbial activities during bioremediation (table 2) [18-20].

Urea which contain 46.6% of nitrogen and NPK of ratio (15:15:15) were the inorganic nutrient used in this study. However if high concentration of the limiting nutrient is applied to the bioremediation soil it can inhibit microbial activity thus the need for optimum CNP ratio is required [21].

To achieve a desirable CNP ratio is important to consider bioremediation optimization [21,22]. In this study CNP ratio 100:2:0.2 was aborted and it gives 88% degradation after 36 days remediation was recorded. This shows that there was a high reduction of TPH in the soils (figure 9).

Total heterotrophic bacterial (THB) and Total hydrocarbon utilizing bacteria (THUB) increased from day 0 to day 18 in the treated group designated NPK and Urea combined (figure 8 and 7). Total heterotrophic fungi (THF) and total hydrocarbon utilizing fungi (THUF) rapidly increased from day 0 to day 12 in the treated group (figure 6 and 5). This result is in agreement with other reports that bio augmentation and bio stimulation lead to increase in microbial population, In this study total hydrocarbon utilizing bacterial and total heterotrophic bacterial in the remediated soil were higher when compare with the unremediated soil [20,22,23]. This indicates that the hydrocarbon utilizing bacteria in the soil was relatively adequate for bioremediation and they line with observation obtained by Hossain et al., 2022 [24]. The findings obtained from the study showed that bio-stimulation of hydrocarbon polluted soil with nutrient amendments induced shifts in the bacterial community with concomitant degradation of the hydrocarbons.

The contaminated soil has higher values for electrical conductivity, bulk density and organic carbon when compared to the treated soil (table 1) which is line with the values obtained by Barua et al., (2011) [25]. Other properties like, porosity, total nitrogen and available phosphorus where higher in the soil treated with urea and NPK (table 2) combine when compared to the contaminated sample (table 1). Crude oil in the soil is known to affect the properties of the impacted soil [26].

The catalase activities of contaminated soils (3000 ppm, 5000 ppm and 8000 ppm crude oil) were monitored. In all the soils (irrespective of the volume of contaminant), it was observed that there was a significant ($P<0.05$) decreased in the activity of catalase in the crude oil soil, this reduction was pronounced in the contaminated soil with no treatment (figure 3). However, as bioremediation proceeded from days 0 to 36, there was resurgence in catalase activity especially in the soils treated with urea and NPK combine (figure 3). This finding corroborates with that of, which found that there was altered activity of soil catalase activity when petroleum products were added to the soil, however, its activity increased few days later which they predicated on the increased microbial activity towards biodegradation of available petroleum hydrocarbon [27]. Also asserted that the initial reduction of catalase activity could be because being an enzyme its activity is altered by unfavorable conditions, such as hypoxia, unavailability of nutrient and changes in PH [28]. This finding place catalase a useful biomarker use for indicating the onset of biodegradation process as their activities decline after the rate of biodegradation has decreased [29].

Lipase concentration increased ($P<0.05$) as the volume of crude oil (contaminant) increased, however, the increased was more pronounced in the samples remediated with urea and NPK combine compared to unremediated soil (figure 4). This finding corroborate with that of where it was found that increasing contaminant concentration increased microbial extracellular lipase activity, thus making lipase as a good option for study of contaminated soil bioremediation [30,3]. Lipase activity has also been reported to be the reason behind the drastic reduction of total hydrocarbon from contaminated soil and its activity has been found to be a very useful indicator parameter for testing hydrocarbon degradation in soil [31,32]. Lipase degrades lipids and other lipid-like compounds derived from a large variety of microorganisms, animals and plants. Lipases can catalyze various reactions such as hydrolysis, inter-esterification, esterification, alcoholysis and aminolysis of organic pollutants laying credence to their avowed role in bioremediation [32].

Laccase and peroxidase are key extracellular oxidative enzymes which are produced by oleophilic microbes [33]. Laccase and peroxidase activities were assayed in this study and a negative relationship was established between them (oxidative enzymes activity) and TPH. This indicate that increase in oxidative enzyme activity leads to decrease in TPH among treated groups (figure 1and 2).

This study also showed that combination of NPK and urea fertilizer of CNP ratio 100:2:0.2 highly affect the TPH value degradation rate in oil contaminated soil (figure 9).

Conclusions

This study established that combination of urea and NPK consequences to 88% degradation of hydrocarbons compared to 68.54% obtained when only NPK was applied as mentioned through different researchers. The impact of bio-stimulation via the utilization of inorganic vitamins and management of parameters like aeration and moisture content confirmed that indigenous soil micro-organism respond nutrient modification evidenced by way of elevated counts recorded in the amended remedies and removal of hydrocarbons. The end result of this learn about also suggests that environmental conditions and the C:N:P ratio adopted shortened the time body for bioremediation of crude oil towards when solely NPK used to be utilized at 10% as suggested by means of different researchers.

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