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Properties of Biostimulant of *Ponderosa* Lemon for Crude Oil Remediation in Soil Environment

Ukpaka C.P.[1], Joel Hellen Chijioke[2,*], Etim Victor Ita[3]

Abstract

The effect of petroleum hydrocarbon on soil environment contributes to low performance of microbes and plant yield. This study investigates the potential of grape leaf (Ponderosa lemon) on hydrocarbon constituents of crude oil contaminated soil. Freshly harvested grape leaf was washed thoroughly with tape water thereafter rinse with distilled water, some portions kept for room-dried and another for sun-dried to vary the composition. Fixed masses of loamy soil samples of 2000 g were mixed with masses of 100 mL crude oil with varying masses of grape leaf (biostimulant) of 50 g and 100 g in a plastic container. Some physicochemical properties were evaluated like pH, total organic carbon (TOC), total nitrogen (TN), phosphate (P), potassium (K), electrical conductivity (EC), heterotrophic utilizing bacteria (HUB), and heterotrophic utilizing fungi (HUF) for 42 days to determine the effectiveness of the biostimulant. The nutritional values of the remediants were quite promising as room-dried is composed of pH 6.74, TOC 8.95%, phosphate 0.891 mg/kg, TN 4.94 mg/kg, potassium 178.52 mg/kg, EC 28.63 $\mu S/cm$, HUB 2.4 × 10^2 cfu/g, and HUF 1.73 × 10^2 sfu/g. Sun-dried grape leaf follows same trend but still lower than the room-dried sample. The changes on the available nutrients for remediant subjected into sun-dried revealed that the bio-stimulant depletion was integrated to the potential effect of sunlight.

Keywords: Properties, biostimulant, *Ponderosa* lemon, crude oil, remediation, soil environment

INTRODUCTION

This study addresses the usefulness of *Ponderosa* lemon (grape leaf) as an essential substance that can be used for remediation of contaminated soil environment. However, this study covers experimental and theoretical concepts, the experimental data shall be filled into the mathematical model for the purpose of evaluating and for the determination of the functional parameters [1–3]. *Ponderosa* lemon is classified as one of the fruits located in tropical region of Africa as well as found useful within the Africa countries as food [4]. The usefulness of the leaf has not been studied and considered in area of bioremediation, rather the people of tropical region of Africa are more interested on the fruit part of the *Ponderosa* lemon [5]. They enhance early root growth, leading to increase a creation and moisture content in soil [5–7]. The use of grape leaf (*Ponderosa* lemon) is a novel study and experimentation for soil remediation. Various leaves have been studied and investigated.

The kinetics of petroleum hydrocarbon mitigation using *Mangifera indica* seed component has been studied to enhance remediation of polluted soil environment [8]. The biostimulant (*Mangifera indica*) known as mango and the part used for

***Author for Correspondence**
Joel Hellen Chijioke
E-mail: joel.hellen@ust.edu.ng

[1]Professor, Department of Chemical/Petrochemical Engineering, Rivers State University, Port Harcourt, Rivers State, Nigeria
[2]Research Student, Department of Chemical/Petrochemical Engineering, Rivers State University, Port Harcourt, Rivers State, Nigeria
[3]Lecturer, Department of Chemical/Petrochemical Engineering, Akwa-Ibom State University, Ikot-Akpaden, Akwa-Ibom State, Nigeria

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research is the seed which was evaluated with respect to their performance and their research proved that the seed is a useful component for bioremediation [9]. The available land for agricultural purposes in Niger Delta Area of Nigeria is now limited due to industrial activities involving oil and gas exploration, exploitation and processing as well as pipeline distribution. It is noticed that the activities of theft in pipeline is increasing day-by-day and the rate of soil spill on the available land for agricultural purposes is limited [10].

The effects of crude oil in soil and aqueous environment have been investigated and solution using the application of biostimulants of different types in different soil environment is well established [11]. The crude oil effect resulting in variation on the physiochemical parameters of the soil as well as the microbial activities of the soil have been reported. The nutrient of the soil and the soil characteristics is well influenced as a result of crude oil deposition. The plants make use of available water in the soil for their growths and toxic nature of the crude oil inhibits the plant growth as result of variation in the water concentration which induced the available nutrients in soil [12]. Research on effect of crude oil in the ecosystem has been reported, including the assessment of its toxic nature in the soil which reveals the available nutrient upon the effect of crude oil contamination. However, there are available techniques identified for the purpose of restoration of the nutrients lost due to contamination resulting from crude oil pollution.

Research on the dispersion of crude oil in soil environment influences the soil greatly and if adequate measures are not taken, there is tendency of the underground water receiving the contaminates, as projected and this phenomenon influences the underground water quality of different aquifers [13, 14]. The need to treat a contaminated soil without delay is necessary due to the fact that dispersion occurs if remedy is not applied after spillage.

The aim of applying bioremediation concepts in handling crude oil contaminated soil environment is necessary because of its effectiveness in treatment and restoration of soil integrity. However, it is observed or recorded that the constituent of the products formed after the biochemical processes has occurred yielded friendly substances (products) that enhance the environment or the ecosystem [15–17].

The concept of bioremediation is recommendable because it mitigates pollutant concentration by the action of microorganism. Indeed, the nutrient of the soil is being improved and the soil nutrient concentration restored for agricultural utilization [18, 19]. The process of bioremediation is well practiced in Nigeria and other parts of the world as a measure to mitigate contaminants concentration and its toxicity.

MATERIALS AND METHODS

Materials

Materials and Experimental Equipment

This study covers the use of the following materials, which includes: loamy soil, crude oil, biostimulator (*Ponderosa* lemon), plastic containers (samples), gas chromatograph (GC) equipment; pH meter, reagents, funnels electronic balance, beaker, desiccators, flasks, sieves mechanical machine, hand towel, oven, thermometer, and equipment to measure electrical conductivity.

Methods

Samples Collection and Analysis

Samples were collected and gas chromatograph was used to determine the total petroleum hydrocarbon concentration as well as the analysis of the physiochemical properties and microbial count was determined as well. The obtain data was translated into mathematical language. The kinetic parameters were determined from the data that was obtained from this research work.

Soil Sampling Parameters

The pH of the soil was determined by subjecting 20 g into 50 mL of water (distilled) and both mixed in a beaker. A modern pH meter of Hanna model of 8614 was subjected into the mixture for the purpose

of determination of the pH value of the soil sample. This was achieved by using filter paper and funnel to obtain a liquid filtered out from the mixture. Tips of the glass and reference electrodes were immersed into the sample for a period of 5 minutes and a constant reading was achieved and the pH meter then calibrated with buffer solution of pH value of 4, 7, and 10.

Conductivity

The soil sample for measuring electrical conductivity was prepared in terms of saturated soil paste with the aid of 100 g or 0.1 kg of 2 mm sieve, the process was air dry of soil sample and the mixture will receive distillated water and the solution was spatulated to achieve the paste glister. The process will allow the spatula being free off excluding the soils containing the plastic loamy solution of paste and allowed it for a period of 1 hour to stand and funnel with filter paper for purpose of filtering the water to achieve only soil sample as extract. At this stage 0.02 mL was used to bridge the electrical conductivity as well as calculate the cell constant, measure and record the reading.

Organic Carbon

Soil sample was collected and size reduced to allow the soil sample pass through the sieve of 0.5 mm and weight 1.0 g, which was introduced into 500 mL Erlenmeyer flask. 1.0 mL of potassium heptaoxochromate (VII) was collected and introduced into a flask and the mixture was swirled gradually for the purpose of separation of the soil sample from the mixture. The soil sample to be prepared received 20 mL of concentrated tetraoxosulfate (VI) and the mixture was subjected into a measuring cylinder for the purpose to achieve a certain volume in the flask. The flask was subjected to swirling gently to achieve proper mixing for a period of 60 seconds and the obtained mixture was subjected into sheet of asbestos which was presented the stand of the flask for a period of 1800 seconds (30 minutes). The mixture received the addition substance of 300 mL of distilled water and 25 mL of iron (II) teteraoxosuphate (VI) and suspension observed as standard potassium teteraoxomanganate (VII) obtained from the burette was achieve by the mechanism of illumination observed with bulb lamp and the process was then titrated. The process allows color change observed from deep green to view purple red. The process also allows the blank examination of the soil and the same procedures were followed and the evaluation procedures as stated.

Calculation of Percentage of Organic Carbon

$$\text{TOC} = \text{M} - \text{N} \text{ and } \text{TB} = \text{L} - \text{M} \tag{1}$$

$$\text{N}(\text{TOC} - \text{TB}) \tag{2}$$

$$\text{w grams of soil contain} = \text{T}(\text{OC} - \text{B}) \times 3 \text{ mg carbon} \tag{3}$$

$$100 \text{ grams of soil contain} = \frac{(\text{TOC}-\text{B})\times 3}{\text{W}} \times \frac{100 \text{ g}}{1000} \text{ carbon} \tag{4}$$

$$\% \text{ organic carbon in the soil} = \frac{(\text{TOC}-\text{B})}{10 \text{ W}} \tag{5}$$

The recovery factors of the above expression are presented as

$$\text{True \% organic carbon in the soil} = \frac{(\text{TOC}-\text{B})\times 3}{10 \text{ W}} \times 1.724 \tag{6}$$

Total Phosphorus in Soil (Bray and Kurtz Methods)

The determination of the total phosphorus soil was carried out using the following procedures of weighing 2.85 g of soil as well as mixing it with 20 mL solution. The obtained solution was stored and also allowed to settle. The solution of the mixture was filtered with funnel and then 10 mL of solution was removed and introduced into another 50 mL volumetric flask with the addition of distilled water as well as 4 mL of B reagent measured. The solution shows color development after 15 minutes. The solution standard set was introduced to different stock for the purpose of achieving the result and the order of stock was 1, 2, 3, 6 and 8 mL of the solution concentration of the standard. Furthermore, the

standard solution which contains 10 mL of the extracted solution as well as the reagent B of 4 mL. The solution color changed after 15 minutes and absorbance in terms of standard was determined.

Curve was obtained by plotting standard of absorbance versus concentration. The absorbance in terms of soil extract was determined as well as phosphorus concentration with reference to the standard curve. The procedure for the calculation is as follows.

Calculation

Considering phosphorus (P) concentration in terms of soil extract (diluted) = Yppm (7)

Phosphorus concentration in terms of soil extract underdilluted = $^{50}/_{10}$ Yppm (8)

Amount of phosphorus in terms of 20 g undilluted of extract = $^{50}/_{10}$ Y × 20 ml (9)

2.85 g of soil was identified

Therefore, 1.0 g of soil contain = $\frac{50}{10}y \times \frac{20}{2.85}\mu g$ (10)

Therefore, the available P in the soil = $\frac{50}{10}y \times \frac{20}{2.85}ppm$ (11)

Soil: The Total Nitrogen Determination (Kjeldahl Digestion and Distribution) Methods

The procedure involves the weighing of 10 g of soil which was air dried and further subjected to grinding to particle size of 0.5 mm which was achieve using sieve and the sample prepared was introduced into 500 mL Kjeldahl flask and 20 mL of distilled water was introduced into the flask and the mixture swirled for some minutes and the mixture was allowed to settle for 1800 seconds. 11.0 g of potassium tetraoxosulphate (VI) in the presence of catalyst mixture in combination with 30 mL solution of the concentrated tetraoxosulphate (VI) acid was heated in a flask at low temperature. The water in the flask was heated to obtain an increase in heating process and this will result to clean solution obtain in the digest. The solution mixture was subjected into boiling for a period of 5 hours and the flask subjected to rotation at time intervals. The solution mixture is further subjected into heating with the addition of the tetraoxosulphate (VI) condensed on the process (middle way) and the flask was subjected to cooling as well as 10 mL of H_2O introduced in the process. The digest solution was poured into a 1-L Erlenmeyer flask and the suspended particles were absorbed in the process. The remaining sand was washed with 50 mL distilled water four times repeatedly and the aliquots were transferred into another Erlenmeyer flask. A solution of 50 mL H_3BO_3 was introduced into Erlenmeyer flask of 500 mL. The solution then was introduced to another Erlenmeyer flask, which was subjected into another Kjeldahl flask. 150 mL of NaOH was allowed to pass through the top to down the neck of the flask and the process was connected to a distillation column. The process allows proper mixing by stirring as distillation commences as well as cooling and the amount of heat controlled for the purpose of mitigation of frothing as well as to avoid suck-back process. Furthermore, 150 mL of distillate was achieved and the flask accumulator was removed and the distillation process stopped.

The ammonium ion in terms of concentration as obtained in the distillate as well as standard tetraoxosuphate (VI) acid was titrated. The process will reveal color change as an indicator to the end of process and the system demonstrates color change of green to pink as the end point. The method for calculation is shown below.

Calculation of Total Nitrogen

Considering W_s gram to be weight in terms of soil used
N defines the normality in terms of H_2SO_4

Therefore, the volume of H_2SO_4 corrected = $(T_s - T_B)$ mL (12)

Amount in terms of H_2SO_4 used = $N(T_S - T_B)$ (13)

Amount in terms of NH_3 distillate = $N(T_S - T_B)$ mg (14)

Amount in terms of N distillate = $N(T_S - T_B)$ mg (15)

Therefore, amount of distillate = $N(T_S - T_B) \times 14$ mg (16)

$$= \frac{N(T_S - T_B) \times 14g}{1000} \quad (17)$$

Thus, W_s g of soil contain can be expressed as:

$$= \frac{N(T_S - T_B) \times 14g \ of \ N}{1000} \quad (18)$$

$$1000 \text{ g of soil contain} = \frac{N(T_S - T_B) \times 100g \ of \ N}{1000 \times W_S} \quad (19)$$

% of total N in the soil

$$\frac{N(T_S - T_B) \times 14 \times 100}{1000 \times w} \quad (20)$$

RESULTS AND DISCUSSION

Characterization of the Physicochemical Properties of Grape Leaf, Soil, and Crude Oil before Contamination

The results of some physiochemical parameters of the samples are presented in Tables 1 to 3. The nutrient composition of grape leaf room-dried (GLRD) showed that total organic carbon 8.95%, potassium 178.52 mg/kg and phosphate 0.891 mg/kg showed favorable conditions for plant growth. Similarly, the presence of hydrocarbon utilizing bacteria and fungi were higher in the GLRD compared to the grape leaf sun-dried (GLSD). The microorganisms present in the crude used as pollutant were isolated and identified as monitored in terms of their growth as the biostimulants were added in each bioreactor set-up. The microbial concentration of the hydrocarbon utilizing bacteria and hydrocarbon utilizing fungi present in the loamy soil was determined in terms of population Similar trend was observed in EC and pH level of the bio-stimulant. The initial total petroleum hydrocarbon (TPH) concentration 48291.14 mg/kg in the crude oil was above the threshold of Nigerian upstream petroleum regulation commission (NUPRC, 2018) (Table 2). The physical properties of the uncontaminated soil in term of soil type indicated that the soil is composed of 40% sand, 20% clay and 40% silt, corresponds to loamy soil as presented in Table 3. However, the same raw material of the biostimulant containing different concentration after analysis of the samples. Indeed, the variation in the concentration of the bio stimulant can be attributed to environmental conditions of sample preparation.

Determination of TPH Degradation under Varying Dosages of Grape Leaf Room-Dried (GLRD) at Different Time Intervals

The results revealed the concentration of TPH with varying dosage of GLRD with contact time. There was reduction on TPH concentration using 50 g GLRD at 7 to 35 days corresponding to 44769.43, 43068.26, 41159.89, 31717.63, and 25305.11 mg/kg with the most reduced at a contact time of 42 days amounting to 19016.73 mg/kg compared to the baseline before biostimulant addition. As compared with the investigation on biostimulated *Vernonia amygdalina* mix with 100 mL spent engine oil contaminated soil amounted to 88.0% reduction of TPH. The obtained result demonstrated the usefulness of *Mangifera indica* seed in the degradation of crude oil contaminated soil. The biostimulant on the specific rate of TPH with varying dosages at contact with the soil and crude oil mixture in a plastic container was monitored and the behavior of each system is illustrated in Figure 1.

Figure 1 showcases the trend of TPH concentration upon the effect of time with the application of 50 g and 100 g dosage of the sun-dried bio stimulant. The effectiveness of the bio stimulant was tested by comparing the performance of the bio stimulant with control system which does not receive any bio stimulant for the purpose of improving the mixture nutrient. Result showcases decrease in concentration of TPH with increase in time.

Table 1. Analysis result of physicochemical parameters of biostimulant (grape leaf) and microbial population.

Sample	GLRD (Remediant)	GLSD (Remediant)
pH	6.74	6.59
Total organic carbon (%)	8.95	6.31
Phosphate (mg/kg)	0.891	0.543
Total nitrogen (mg/kg)	4.94	3.77
Potassium (mg/kg)	178.52	124.19
Electrical conductivity (μS/cm)	28.63	17.98
Heterotrophic utilizing bacteria (cfu/g)	2.4×10^2	1.35×10^2
heterotrophic utilizing fungi (cfu/g)	1.73×10^2	1.02×10^2

Note: GLRD = grape leaf room-dried; GLSD = grape leaf sun-dried.

Table 2. Analysis result of crude oil (pollutant).

Sample	Crude oil
pH	6.76
Pour point (°C)	-12.8
Density at 15°C (g/mL)	0.878
Kinematic viscosity at 40°C (mm^2/s)	147.4
Flash point (°C)	193.2
Total petroleum hydrocarbon (ppm)	48291.14

Table 3. Analysis result of the USDA (US Department of Agriculture) soil texture classification.

Sample Parameter	Soil
Sand (%)	40
Clay (%)	20
Silt (%)	40
Textural class	*Loamy soil*

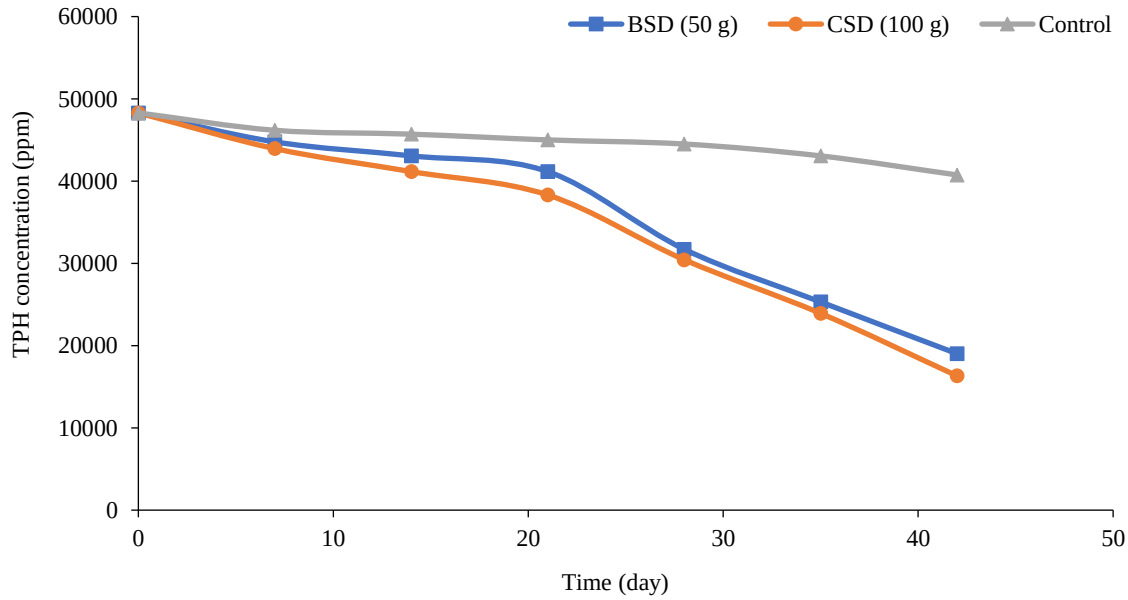

Figure 1. Total petroleum hydrocarbon (TPH) concentration against time for sun-dried application of biostimulant and control.

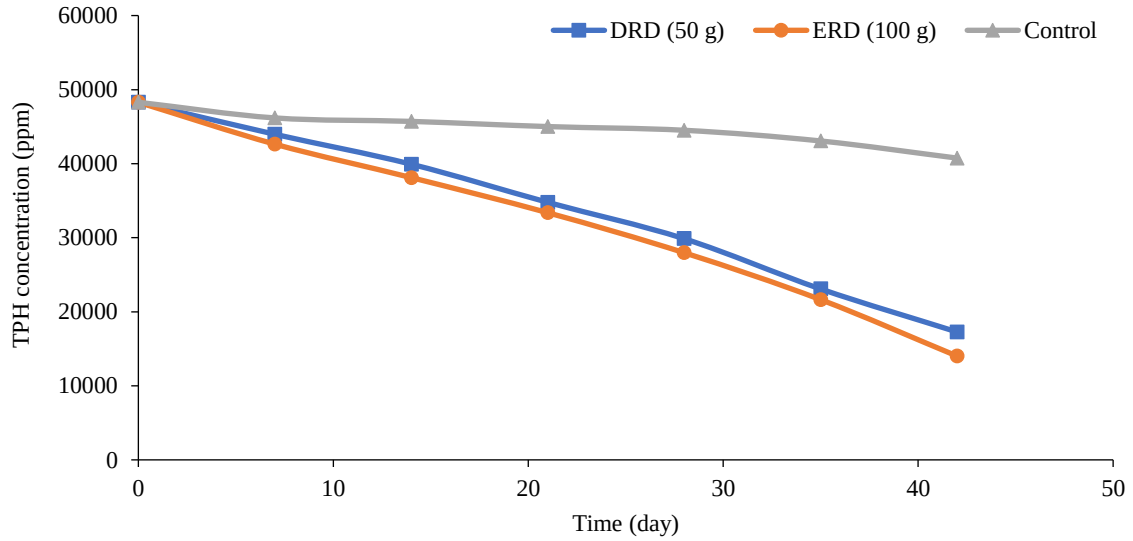

Figure 2. Total petroleum hydrocarbon (TPH) concentration against time for room-dried application of bio stimulant and control.

Figure 1 further revealed high remediation in the bioreactor containing 100 g dosage of the sun-dried biostimulant than 50 g dosage of biostimulant. As compared with respect to the case of 30 g of mango leaves sample at 28 days treatment, there was considerable reduction of TPH amounting to 93.40%. This suggests that increased application of GLSD contributed to reduction of TPH.

Figure 2 showcases the trend of TPH concentration upon the effect of time with the application of 50 g and 100 g dosages of the room-dried biostimulant. The effectiveness of the biostimulant was tested by comparing the performance of the bio stimulant with control system which does not receive any biostimulant for the purpose of improving the mixture nutrient. The result showcases decrease in concentration of TPH with increase in time. Figure 2 further revealed high remediation in the bioreactor containing 100 g dosage of the room-dried bio stimulant than 50 g dosage of bio stimulant.

CONCLUSIONS

The bioremediation potential of sun-dried and room-dried grape leaf over a period of 42 days with a view of assessing their suitability in the rate of degradation of petroleum hydrocarbons in the soil was investigated. The results obtained from this study supports the following conclusions.

The chemical and microbiological properties of the sun-dried and room-dried grape leaves before ther were utilized as treatment, were investigated. The nutritional values of the remediants were quite promising as room-dried is composed of pH 6.74, total organic carbon (TOC) 8.95%, phosphate 0.891 mg/kg, total nitrogen (TN), 4.94 mg/kg, potassium 178.52 mg/kg, electrical conductivity (EC) 28.63 µS/cm, heterotrophic utilizing bacteria (HUB) 2.4×10^2 cfu/g, and heterotrophic utilizing bacteria (HUF) 1.73×10^2 cfu/g. Sun-dried grape leaf follows same trend but still lower than the room-dried grape leaf. The obtained information from the research has shown that the properties of the available nutrients and the condition of preparation influence the nutrients concentration of the useful substances that catalyzes the reaction mechanism for the microbial build-up during bioremediation process of crude oil degradation.

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