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Research Article

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Bioremediation of Petroleum Contaminated Soils Using Fresh Lemon Grass (*Cymbopogon citratus*) Granules

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Abstract This work is aimed at examining the use of fresh lemongrass granules as a local raw material in the treatment of petroleum contaminated soils in the Niger Delta Region of Nigeria. Composite soil samples were obtained from Oshiobele Community in Ahoada West Local Government Area of Rivers State at a depth of 15cm using the soil auger while poultry droppings and fresh lemongrass were collected from Rivers State University Farm. The samples were placed in sterile containers, properly labeled and sent to the laboratory for analyses using standard methods. The determined parameters were soil physicochemical parameters such as Total Petroleum Hydrocarbon (TPH) which was analyzed by using ASTM method D3921, pH was by using ASTM D4972 Hanna H1 2211 pH/ORP Meter, total nitrogen and phosphorus were analyzed using APHA methods and microbiology was carried out using the Cheesbrough method. Soil type classification and particle size analyses revealed that the soil samples were Clay soils. The results obtained at the end of 28 days indicated that Total Petroleum Hydrocarbon decreased from1367.42ppm to 1283.92ppm for soils in A, 1322.11ppm to 801.44ppm for soils in B and 1326ppm to 311.62ppm for soils in C. The soil pH values were 4.52, 4.96 and 6.51 for soils in A, B and C respectively. Also total nitrogen was 0.4, 0.347 and 0.11% for A, B and C. The phosphorous level of the soils in A, B and C at the end of the remediation process were 1.52%, 1.19%, and 0.24% accordingly. The Total Heterotrophic Bacteria count obtained were3.6 x 10^3 cfu/g for the soils. The Hydrocarbon Utilizing Bacteria count in soils in A decreased from 3.65x10^3 cfu/g to 1.9x 10^2 cfu/g but an increase to 4.78x10^3 cfu/g and 5.41x10^3 cfu/g were noticed in soils in B and C respectively. The results revealed that the concentration of Total Petroleum Hydrocarbon decreased by 6%, 39.4% and 77% in soils in A, B and C respectively, which implies that there was significant decrease in concentration of Total Petroleum Hydrocarbon in the soils in C compared to the soils in A and B. This revealed that the use of fresh lemongrass granules alone in the biodegradation of petroleum hydrocarbons is less effective rather, the combination of fresh lemon grass granules and poultry dropping enhanced the growth of microorganism, which made it more effective and efficient method in the treatment of soils contaminated with petroleum hydrocarbons.

Keywords Bioremediation, Lemongrass, Poultry droppings, Soils

Introduction

Bioremediation is a microbiological process applied to break down or transform contaminants to less toxic or nontoxic forms. And it involves degrading, removing, altering, immobilizing, or detoxifying various chemicals and physical wastes from the environment through the action of bacteria, fungi and plants. Microorganisms involved act through their enzymatic pathways as biocatalysts which facilitate the process of biochemical reactions that degrade the desired pollutants. The efficiency of bioremediation depends on many factors which

include, the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their availability to microorganisms [1].

Lemongrass (*Cymbopogon citratus*) is a tall perennial grass, widely cultivated in warm tropical and subtropical regions [10]. It contains 1% to 2% essential oil on a dry basis and its chemical composition varies as a function of genetic diversity, habitat, and agronomic treatment of the culture [9]. The volatile oil obtained from the fresh leaves of this grass is widely used in the perfume and cosmetic industries. It is mostly composed of monoterpene compounds and citral which is a major component, which is a natural mixture of two isomeric acyclic monoterpene aldehydes, geranial and neral. Apart from citral, the oil also consists of myrcene, geraniol, and geranyl acetate [8]. Lemongrass oil does not only damage the membrane structure through monoterpene diffusion but also facilitates solubility in cell membranes when applied in gaseous form [3]. Furthermore, lemongrass essential oil has antidepressant, antioxidant, antiseptic, astringent, nervine, sedative as well as bactericidal, fungicidal, and generally antimicrobial activity against diverse range of microorganisms including moulds and yeasts [2]. With the abundance of lemon grass in the Niger Delta part of Nigeria, it has become imperative to examine its effect as a local raw material in the treatment of petroleum contaminated soils in the Niger Delta being a host to the Oil and Gas activities in Nigeria.

Materials and Methods

Soil Sampling

The soil samples were collected using hand soil auger from Oshiobele Community in Ahoada West Local Government Area of Rivers State. The collected soil samples were bulked together and put into well labelled glass bottles and sealed with aluminum foil, and used for Total Petroleum Hydrocarbon (TPH) analysis [12] while the poultry droppings and fresh lemongrass were collected from the Rivers State University Farm.

Experimental Procedure

The fresh lemongrass was converted into granules using a grinding machine. This was to reduce the surface area of the fresh lemon grass for easy assimilation into the contaminated soils. One hundred and fifty kilogram (150kg) of soil samples were weighed into a bowel and contaminated with 15000mls (15litre) of Bonny light crude oil by using standard pollution volume of 100mls of crude oil to 1kg of soil. The reason was to provide condition of major crude oil spill. The mixture was properly mixed to ensure uniform concentration of the crude oil in the soil samples and left for three days to settle without any disturbance. Thereafter, fifty (50) kilogram each of the contaminated soils were obtained and transferred into three different bowels labeled A, B and C. The treatment of the soils commenced after three days with application and mixing of 1000g of fresh lemongrass granules with the soils in B and C. Furthermore, 450g of poultry droppings were added to the soil samples in C as nutrients, while soil samples in A had no treatment added to the contaminated soils and it served as control for the process. One hundred and fifty (150mls) milliliters of water was sprinkled on the soil samples in A, B and C every two days for 28 days to enhance the moisture content of the contaminated soils. Soil samples were collected from the samples in A, B and C every seven (7) days for analysis in the Laboratory.

Determination of Physicochemical Properties

The soil samples were analyzed for particle size distribution and classification, pH, total petroleum hydrocarbon, total nitrogen, and phosphorous. The physicochemical properties were determined using standard methods adopted from relevant literatures**.** Soil type classification and particle size analyses were carried out before contamination of the soil with crude oil by hydrometer method using sodium hexametaphosphate as the dispersing agent [6]. The soil structural classification was obtained, using the United State Department of Agriculture (USDA, 1987) soil textural classification scheme using TAL®for Windows software. The pH levels of the soil samples were determined in the laboratory using Hanna HI 2211 pH/ORP meter according to ASTM (1999) method D4972. Total Petroleum Hydrocarbon was analyzed by using Gas Chromatograph-Flame Ionization Detector (GC-FID) Model, HP 5890 Series II, U.S.A., after extraction of hydrocarbon content by applying ASTM (1999) method D3921 [13]. Total nitrogen was determined by using APHA (1998) method, 4500-NO3 B while Phosphorous was analyzed by using APHA (1998) method, 4500-$PO43^{-}$. In cultivation of

total heterotrophic bacteria, prepared nutrient agar culture plates were made according to the manufacturer's specification (HIMEDIA) M001-500G, HIMEDIA Laboratories Pvt. LTD Number-400086, India). The culture plates were dried and 0.1ml of the 10^1 diluted soil sample was placed on it using sterile pipette and spread using a sterile glass rod spreader to dryness on the plate. This was incubated in an incubator at 37 ºC for 24 hours and the counting of the bacteria was made on the plate after the bacteria have shown growth. The bacteria that did not grow after 24 hours were further allowed incubated in the incubator for another 24 hours and readings were made on them. The total bacteria count was made and recorded [7]. Also, the Vapour phase technique was used to grow and identify the hydrocarbon utilizing bacteria. The culture plates were prepared by using the Mineral salt agar without the carbon source [11]. The plates were dried and 0.1ml of the 10^1 diluted soil samples were placed on the dried plates. The samples were spread on the minerals salt agar plate using a sterile glass rod spreader to dryness. A crude oil (Bonny light) soaked on ninety (90mmø) millilitre diameter Whatman filter paper No.1 (Whatman International Ltd Maid store, England) was placed on the cover of the cultured plates and were incubated at room temperature for initial three (3 days) with observation and extended to seven (7 days) for extended observation. The hydrocarbon utilizing bacteria were counted during the periods and recorded accordingly. Further tests were carried out to identify the bacteria using Okpokwasili & Odokoma, 1990 techniques. To identify the isolated bacteria, pure cultures of the isolates were prepared by aseptically streaking representative colonies of the different cultures, which appeared on the culture plates, onto dried nutrient agarplates and incubated at 37 ºC for 24 hours. The nutrient agar plates were stored in a refrigerator and this served as pure stock culture for subsequent characterization and identification tests. Standard characterization tests (such as Gram staining, motility, oxidase test, and catalase and other tests) were performed. The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics [7]. The degree of degradation of hydrocarbon was obtained by calculation, using Equation (1) below.

$$\%\, D \quad = \frac{TPHi - TPHf}{TPHi} \times 100 \qquad (1)$$

where TPH_i and TPH_f represent the initial and final concentration of Total Petroleum Hydrocarbon [12].

Results and Discussion

Table 1: Physicochemical Characteristics Before and After Pollution (Result Represent Mean± Standard Deviation of Three Replicates)

Parameters				**TPH (PPM)**	**pH**	**TN (%)**	**P (mg/kg)**	**THB (cfu/ml)**
Before Contamination				6.35 ± 0.02	6.32 ±0.12	0.081 ±0.04	0.15 ±0.23	$5.32x10^3$±0.01
After Contamination				1.482.42 ± 0.09	4.12 ±3	0.560 ±0.08	1.98 ±2	$1.65x10^3$ ± 0.03
PSD	Sand(%)	Silt(%)	Clay(%)	Bulk Density(g/cm^3)		Porosity		
	9.50	15.30	75.20	1.432		0.461		

Key:
TPH - Total Petroleum Hydrocarbon
TN - Total Nitrogen
P - Phosphorus
THB - Total Hydrocarbon Utilizing Bacteria
PSD - Particle Side Distribution

Table 1 shows the results of physicochemical characteristics of the soil before and after pollution with crude oil. Particle Size Distribution (PSD) and soil classification revealed that the soil was Clay soil with 9.50% Sand, 15.30% Silt, 75.20% Clay, 1.432g/cm Bulk density and 0.461 Porosity. Also, the results indicate that there was a change in the initial conditions of the soils after pollution with Crude oil. This is as a result of the introduction of pollutants into the soils by the Crude oil pollution [13].

Variation of Total Petroleum Hydrocarbon with Time

Figure 1 shows the variation of Total Petroleum Hydrocarbon with time for soils sample in A, B and C. The soils in A had contaminated soils which function as the control Sample. The Soils in B had fresh lemongrass granules while C had lemongrass granules and poultry dropping. The graph indicates that there was a decrease in concentration of Total Petroleum Hydrocarbon in the soils in C compared to the soils in A and B.

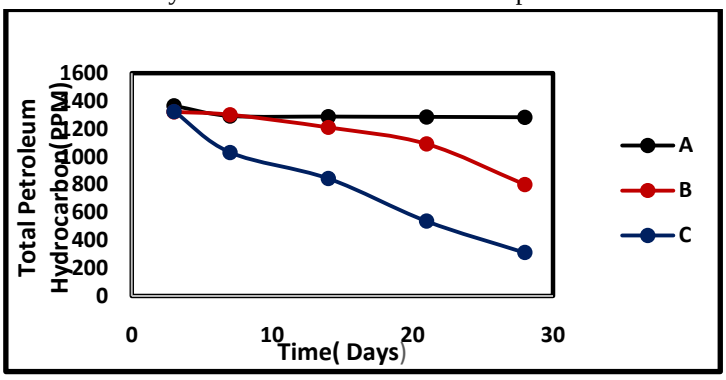

Figure 1: Variation of Total Petroleum Hydrocarbon with Time

The results revealed that the concentration of Total Petroleum Hydrocarbon decreased by 6%, 39.4% and 77% in soils in A, B and C respectively. The result obtained in C maybe as a result of the combination of fresh lemongrass granules with nutrient which encouraged increase in microbial population that depend on the pollutants for energy during biodegradation which reduced the concentration of the contaminants. Statistical evaluation showed significant difference at $P<0.05$ for soil samples in C.

Variation of pH with Time

Figure 2 shows the variation of pH with time for soils in A, B and C. The graph revealed that there was an increase in the acidity levels in soils in A and B while soils in C noticed appreciable decrease in acidity levels. The increasein acidity levels in soils sample A and B were as a result of introduction of pollutants into the soils by the crude oil pollution.

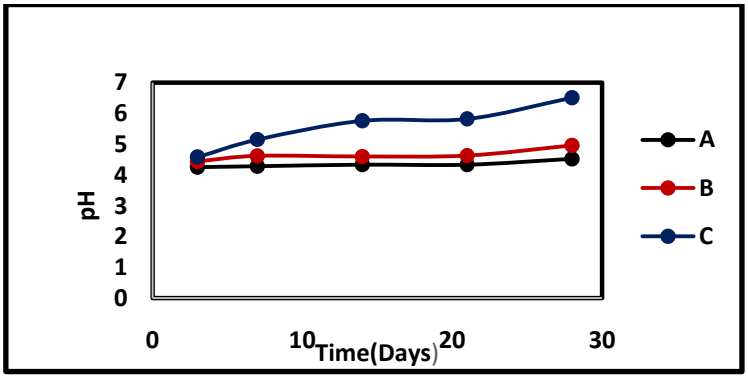

Figure 2: Variation of pH with Time

Also, the results showed less effect on reducing the acidity level of the soils in A as the control while B with the lemongrass as the only treatment. The decrease in acidity levels of soils in C may be due to the effect of applying both fresh lemongrass and poultry dropping which promoted increase in population of hydrocarbon utilizing bacteria thereby reduced the concentration of the contaminants that were responsible for the increase in acidity level of the soils. Statistical evaluation showed significant difference at $P<0.05$ for samples in C.

Variation of Total Nitrogen with Time

Figure 3 shows the variation of total nitrogen with time for soils in A, B and C. It indicates that soils in A and B noticed slight decrease in concentration of total nitrogen compared to the soils in C with significant decrease in concentration of total nitrogen. This is due to depletion in concentration of nitrate in the soils because of crude oil pollution for a certain period of time without replacement of the lost nutrients [13].

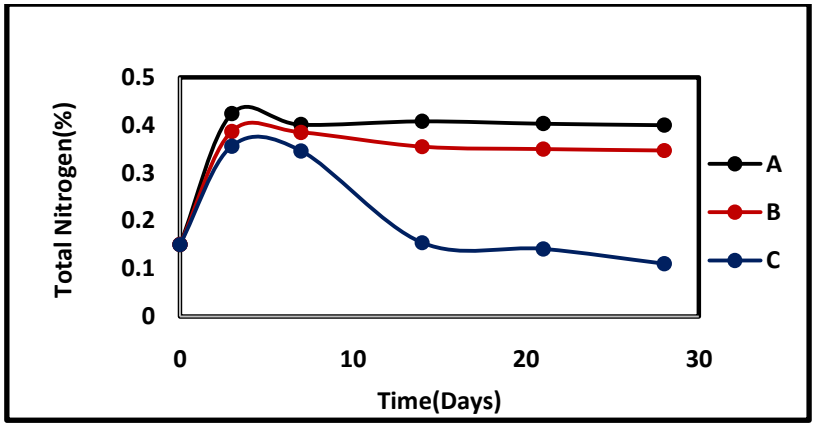

Figure 3: Variation of Total Nitrogen with Time

The significant decrease in the concentration of nitrate in soils in C revealed the effectiveness of combining fresh lemongrass granules and poultry dropping for bioremediation of petroleum contaminated soils. This process enhanced the increase in population of microorganism which gave rise to higher demand of nitrate by the microorganisms as nutrient for biodegradation of hydrocarbons thereby depleting the concentration of total nitrogen in the contaminated soils. Statistical evaluation showed significant difference at $P<0.05$ for soil samples in C.

Variation of Phosphorus with Time

Figure 4 shows the variation of phosphorous with time for soils in A, B and C. The results had similar trend with the results obtained in variation of total nitrogen with time in our earlier discussion above. The result explained that there was a decrease in concentration of phosphorus in the soils in A, B and C, but with appreciable decrease recorded in soils in C. The slight decrease in concentration of phosphorus in soils in A and B may be due to less demand for phosphate as nutrient by the microorganism because of insufficient bacteria to use it as nutrients while the rapid decrease of its concentration in soils in C were as a result of increased population of microorganism which utilized the phosphate as nutrient during biodegradation of hydrocarbons in the soils.

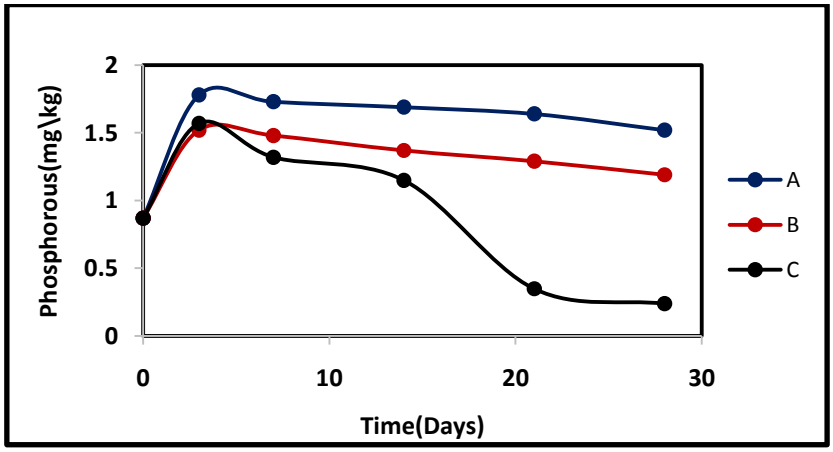

Figure 4: Variation of Phosphorus with Time

This implies that combination of fresh lemongrass granules and poultry droppings enhanced the growth of microorganism used in biodegradation of petroleum contaminants in the soils, therefore, it is an effective and efficient product for bioremediation of petroleum contaminated soils. Statistical evaluation showed significant difference at $P<0.05$ for soil in C.

Variation of Hydrocarbon Utilizing Bacteria with Time

Figure 5, shows the variation of Hydrocarbon Utilizing Bacteria (HUB) with time for soils in A, B and C. The graph revealed that, there was a decrease in population of Hydrocarbon Utilizing Bacteria in soils in A. The

decrease in population of the bacteria in soil sample in A may be as a result of the negative effect of the pollutants from the crude oil on it.

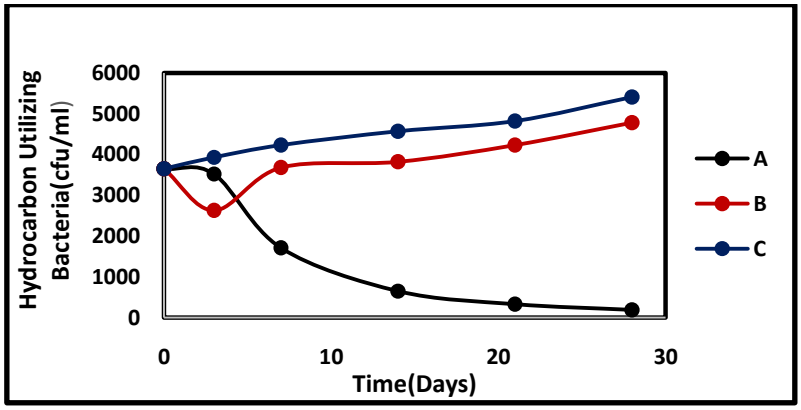

Figure 5: Variation of Hydrocarbon Utilizing Bacteria with Time

Also, soils in B and C experienced an increase in microbial population with soil in C having the highest microbial population growth. This indicates that the combination of fresh lemon grass and poultry droppings support the growth of bacteria which gave rise to an effective and efficient bioremediation of petroleum contaminated soils. Statistical evaluation showed significant difference at $P<0.05$ for Soils samples in C.

Conclusion

The results indicate that the concentration of Total Petroleum Hydrocarbon decreased by 6%, 39.4% and 77% in soils in A, B and C respectively, which implies that there was a significant decrease in concentration of Total Petroleum Hydrocarbon in the soils in C compared to the soils in A and B. Also, it revealed that the use of fresh lemongrass granules alone in biodegradation of petroleum hydrocarbons are less effective rather, the combination of fresh lemongrass granules and poultry dropping enhanced the growth of microorganism, which made it more effective and efficient method in the treatment of soils contaminated with petroleum hydrocarbons. Therefore, it could be recommended that for effective bioremediation to be achieved in crude oil polluted area(s) especially in the Niger Delta area of Nigeria this combination could be encouraged.

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