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Research Article

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Application of Dry Lemon Grass (*Cymbopogon citratus*) Granules on Treatment of Petroleum Hydrocarbon Polluted Soils

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Abstract This work is aimed at examining the use of dry lemongrass granules or its combination with poultry droppings as a local raw material in the treatment of petroleum contaminated soils in the Niger Delta part of Nigeria. The soil samples were obtained with hand soil auger and analyzed in the laboratory using standard methods. The determined parameters were total petroleum hydrocarbon, pH, total nitrogen and phosphorous and hydrocarbon utilizing bacteria. The results obtained at 28 days indicated that the soils samples were Clay soils and total petroleum hydrocarbon decreased from1367.42 ppm to 1283.92ppm for soils sample A, 1358.11ppm to 1106.72ppm for soils sample D and 1328ppm to 1010.16ppm for soils sample E. The soil pH levels were 4.52, 5.0 and 6.10 for soil sample A, D and E respectively. Also total nitrogen was 0.425, 0.398 and 0.355% for A, D and E. In the same vein, the concentration of phosphorous in soil sample A, D and E were 1.52%, 1.32%, and 1.26%. The hydrocarbon utilizing bacteria count decreased from $3.65x10^3$ cfu/g to $1.9x\ 10^2$ cfu/g, in soil sample A, $1.87x10^3$ cfu/g in D and $2.10x10^3$ cfu/g in E respectively. The results revealed that the concentration of total petroleum hydrocarbon decreased by 6%, 19% and 24% in soils samples A, D and E, respectively. This implies that there was a low decrease in concentration of petroleum hydrocarbon in the soils. Also, it indicated that the use of dry lemongrass granules in the biodegradation of petroleum hydrocarbons is not effective. The combination of dry lemon grass granules and poultry dropping was expected to enhance the growth of microorganism that will promote the biodegradation of petroleum hydrocarbons, but a very minimal outcome was obtained, hence this combination did not provide a good support to the bioremediation process. The results conclude that the use of dry lemongrass or its combination with nutrient (Poultry droppings) is less effective in the treatment of soils contaminated with petroleum hydrocarbons.

Keywords Biodegradation, Dry lemongrass, Hydrocarbons, Pollutants

1. Introduction

Bioremediation is a process that applied the use of microorganisms to break down or transform pollutants to less toxic or nontoxic forms [1]. It involves transformation of the chemical and physical properties of the pollutants through the action of microorganism. 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 such as, the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their availability to microorganisms [2]. Bioremediation is effective in an environment with sufficient oxygen. It is only in rare cases that biodegradation occurs in anoxic environment [2]. Biodegrading bacteria require sufficient oxygen for breakdown of petroleum

hydrocarbons [3]. Also, one of the major factors for effective bioremediation of petroleum hydrocarbon is the availability of nutrients. The most essential nutrients are nitrogen and phosphorous. The application of nitrogen and phosphorous increases the proliferation of biodegrading bacteria, resulting in an increase in degradation rates [4]. Lemongrass (*Cymbopogon citratus*) is a tall perennial grass, widely cultivated in warm tropical and subtropical regions [5]. 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 [6]. The results of recent work have revealed that the application of fresh lemongrass granules and poultry droppings on biodegradation of petroleum hydrocarbon is very effective [7]. In the same vein, it will be appropriate to examine its dry effects as a local raw material in the treatment of petroleum contaminated soils in the Niger Delta area as a host to oil and gas related activities.

2. 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 [8], while the poultry droppings and dry lemon grass were collected from the Rivers State University Farm [7].

Experimental Procedure

Thirty kilogram (30 kg) of fresh lemongrass were dried at room temperature for 14days. The dry lemon grass was converted into granules using a grinding machine. This was to reduce the surface area of the dry lemon grass for easy absorption by the contaminated soils [7]. 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 [8]. The reason was to showcase a 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, D and E. The treatment of the soils commenced after three days with application and mixing of 1000g of dry lemon grass granules with the soils in D and E. Furthermore, 450g of poultry droppings were added to the soil samples in E as nutrients, while soil samples A had no treatment added to the contaminated soils. This served as control for the process. One hundred and fifty (150mls) milliliters of water was sprinkled on the soil sample A, D and E every two days to enhance the moisture content of the contaminated soils. Soil samples were collected from soil samples A, D and E every seven (7) days for analysis in the Laboratory [7].

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 [9]. 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 [10] method D3921[11]. Total nitrogen was determined by using APHA [12] method, 4500-NO3 B while phosphorous was analyzed by using APHA [12] 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^{O}C for 24hours 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 24hours and readings were made on them. The total bacteria count was made and recorded [13]. 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 [14]. The plates were dried and 0.1ml of the 10^1diluted 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 (3days) with observation and extended to seven (7days) 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, [14] 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 agar plates 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 [13]. The degree of degradation of hydrocarbon was obtained by calculation, using Equation (1) below.

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

where TPH_i and TPH_f represent the initial and final concentrations of Total Petroleum Hydrocarbon [8].

3. 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)**	**HUB (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: Total Petroleum Hydrocarbon (TPH), Acidity level (pH), Total Nitrogen (TN), Phosphorus (P), Hydrocarbon Utilizing Bacteria (HUB) and Particle Side Distribution (PSD).

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 [7].

Variation of Total Petroleum Hydrocarbon with Time

Figure 1 shows the variation of Total Petroleum Hydrocarbon with time for soil sample A, D and E. The graph shows that there was a slight decrease in concentration of total petroleum hydrocarbon in soil sample E compared to soil sample A and D.

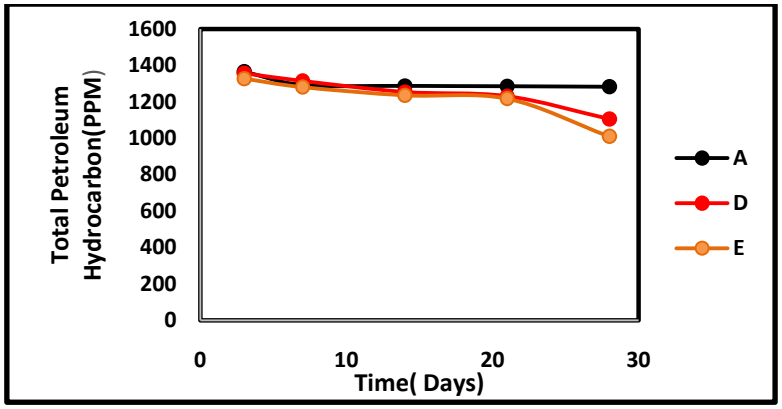

Figure 1: Variation of Total Petroleum Hydrocarbon with Time

The results revealed that the concentration of total petroleum hydrocarbon decreased by 6%, 19% and 24% in soil sample A, D and E respectively. The result obtained in E may be as a result of the combination of dry 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 no significant difference at P<0.05 for soil sample E.

Variation of pH with Time

Figure 2 shows the variation of pH with time for soil sample A, D and E. The graph revealed that there was an increase in the acidity levels in soils in A and D while soils in E noticed appreciable decrease in acidity levels. The increase in acidity levels in soils sample A and D were as a result of introduction of pollutants into the soils by the crude oil pollution (Umeda *et al.*,2021).

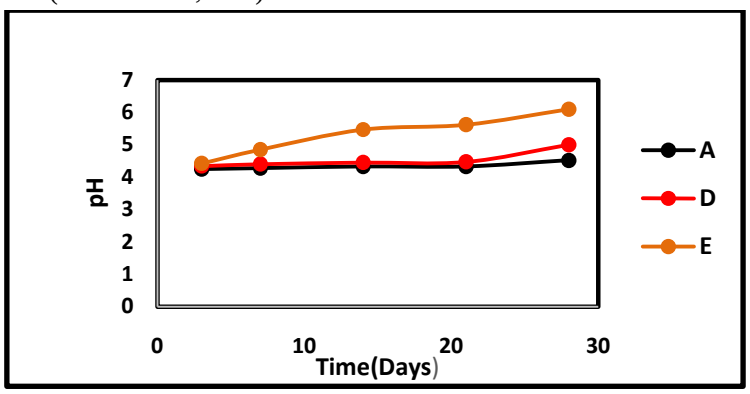

Figure 2: Variation of pH with Time

Also, the results showed less effect on reducing the acidity level of the soils in A as the control while D with the lemon grass as the only treatment. The decrease in acidity levels of soils in E may be due to the effect of applying both dry lemongrass and poultry dropping which slightly improved the acidity level of the soils. Statistical evaluation showed no 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 soil sample A, D and E. It indicates that soils sample A, D and E had slight decrease in concentration of total nitrogen.

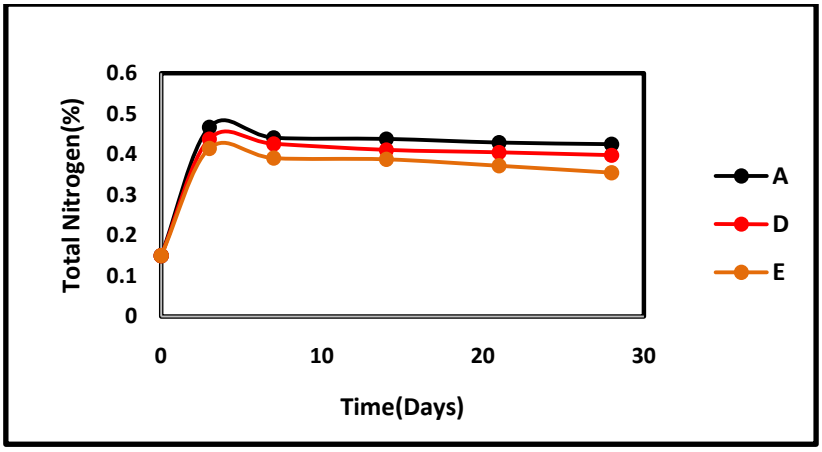

Figure 3: Variation of Total Nitrogen with Time

The slight or low decrease in the concentration of nitrate in the soils samples (A, D and E) revealed that the use of dry lemongrass granules or its combination with poultry dropping for bioremediation of petroleum contaminated soils is less effective. This implies that the process did not support the growth in population of microorganisms which led to very poor demand of nitrate by the microorganisms as nutrient for biodegradation of petroleum hydrocarbons. Statistical evaluation showed no significant difference at $P<0.05$ for soil samples E.

Variation of Phosphorous with Time

Figure 4 shows the variation of phosphorous with time for soil sample A, D and E. The result followed the same trend with the results obtained in variation of total nitrogen with time. The result explained that there was a slight decrease in concentration of phosphorous in soil sample A, D and E. The slight decrease in concentration of phosphorus in soil sample A, D and E may be due to less demand for phosphate as nutrient by the microorganisms because of its low population.

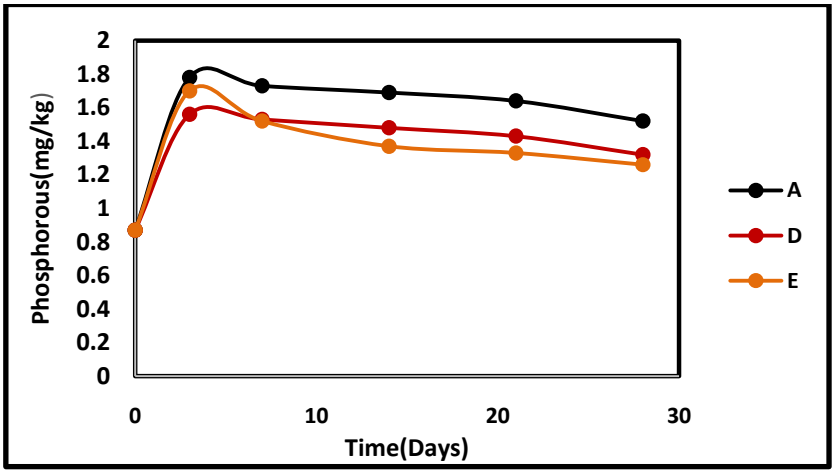

Figure 4: Variation of Phosphorous with Time

This implies that the applied method did not enhance the growth of microorganism responsible for biodegradation of petroleum contaminants in the soils. Statistical evaluation showed no significant difference at $P<0.05$ for soil in E.

Variation of Hydrocarbon Utilizing Bacteria with Time

Figure 5, shows the variation of Hydrocarbon Utilizing Bacteria (HUB) with time for soil sample A, D and E. The graph revealed that, there was a decrease in population of hydrocarbon utilizing bacteria in soils samples. The bacteria count decreased from $3.65x10^3$ cfu/g to $1.9x\ 10^2$ cfu/g, in soil sample A, $1.87x10^3$ cfu/g in D and $2.10x10^3$ cfu/g in E respectively. The decrease in population of the bacteria in the soil samples may be due to the negative impact of the pollutants (Crude Oil) on the soils.

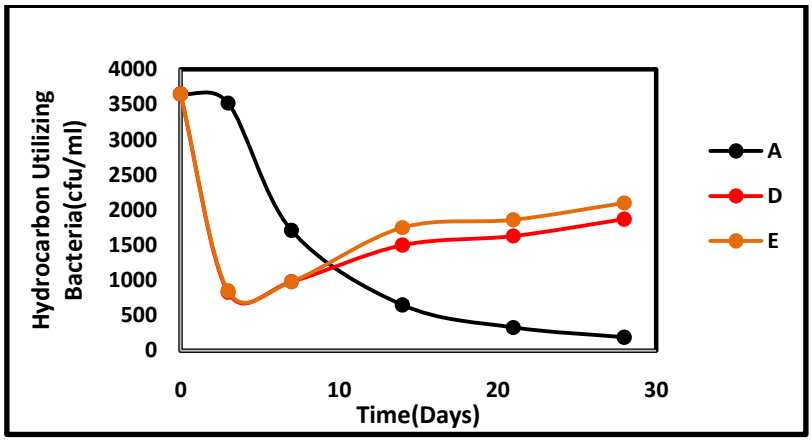

Figure 5: Variation of Hydrocarbon Utilizing Bacteria with Time

This shows that dry lemon grass or its combination with poultry droppings did not provide the best support needed in the growth of microorganisms. Statistical evaluation showed no significant difference at $P<0.05$ for Soils samples in E.

4. Conclusion

The results indicate that the concentration of total petroleum hydrocarbon decreased by 6%, 19% and 24% in soil sample A, D and E, respectively, which implies that the process experienced slight decrease in concentrations of total petroleum hydrocarbon. It further explained that the use of dry lemongrass granules or its combination with poultry dropping in biodegradation of petroleum hydrocarbons are less effective. This is because both process did not support rapid growth of microorganisms, hence a decline in the process of biodegradation of petroleum hydrocarbon was experienced, which confirmed that the process is not the best method in the treatment of soils contaminated with petroleum hydrocarbons.

References

[1]. Jelena, M.S., Beskoski, V.P., llic, M.V. & Ali, S.A.M (2009). Bioremediation of soil heavy contaminated with crude oil and its products. Composition of microbial consortium. Journal of the Serbian Chemical Society, 74, 455 – 460.

[2]. Abatenh E., Gizaw B., Tsegaye Z., Wassie M. (2017). The role of microorganism in bioremediation – A Review. *Open Journal of Environmental Biology*, 2, 038 – 046.

[3]. Atlas, R.M & Philip, J. (2005). Bioremediation. applied microbial solution for real –world environment cleans up. *ASM Press*.

[4]. Resenberg, E., Legmam, R., Kushmaro, A., Adler, E., Abir, H. & Ron, E.Z. (1996). Oil bioremediation using insoluble nitrogen source. Journal of Biotechnology 273 -278

[5]. Naik, M.I. Fomda, B.A. Jaykumar, E & Bhat, J.A. (2010) Antibacterial activity of lemon grass (*cymbopogon citratus*) oil against some selected pathogenic bacteria. *Asian Pac. J. Trop. Med*. 3, 535–538

[6]. Carlson, L.H.C. Machado, R.A.F. Spricigo, C.B. Pereira, L.K & Bolzan, A. (2001). Extraction of lemongrass essential oil with dense carbon dioxide. *J. Supercrit. Fluids*, 21, 33–39.

[7]. Uchendu Umeda, Emmanuel O. Ehirim, Ollor A. Ollor & Happiness Orlu (2021). Boiremediation of petroleum contaminated soils using Fresh lemongrass (*Cymbopogon citratus*) granules. Journal of Scientific and Engineering Research,1.167 -173

[8]. Umeda, Uchendu & Ollor, Ollor Amba (2019). Impact of oxygen diffusion on indigenous bacteria at 100cm depth for bioremediation of hydrocarbon contaminated soils. *International Journal of Research in Engineering and Science*, 3, 24-30.

[9]. Ayotamuno, J.M., Okparanma, R.N. & Amadi, F. (2011). Enhanced remediation of oil sludge with saline water. *African Journal of Environmental Science and Technology*, 5, 262-267.

[10]. American Society for Testing & Materials (ASTM) (1999). Water (II). Volume 11.02. 100 Barr Harbor Drive, P.O. Box C700, West Conshohocken, 19428 – 2959.

[11]. Uchendu Umeda, Emmanuel O. Ehirim & Kenneth K. Dagde (2019). Effect of Oxygen Diffusion on Physicochemical Characteristics of Petroleum Contaminated Sandy Loam Soil. *IOSR Journal of Engineering*, 4, 23-31.
[12]. American Public-Health Association (APHA) (1998). Standard methods for the examination of water and wastewater (20th ed). Washington DC.
[13]. Barrow, G I. & Feltham, R. K. A. (2003). *Cowan and steel's manual for the identification of medical Bacteria.* (3rd) Edition. Published by the Press Syndicate of the University of Cambridge, Cambridge, United Kingdom.
[14]. Okpokwasili, G.C. & Odukoma, L.O. (1994). Tolerance of nitrobacter to toxicity of some nigerian crude oil. *Bull. Environ. Contan. Toxicol*, 52, 388-395.