EFFICACY OF AMENDMENTS IN TPH REMOVAL DURING BIOREMEDIATION OF AGRICULTURAL CRUDE OIL-POLLUTED SOIL.

Abstract

In this study, we set out to identify the efficacy of different amendments as biostimulants in bioremediation and choose the best for our soil type and contaminant. The amendments used were poultry dropping (C-PD), cow dung (D-CD), N.P.K (E), and a control (A and B) setup undergoing natural attenuation. The microcosms were monitored for physicochemical characteristics such as total organic nitrogen, pH, temperature, total organic carbon, total petroleum hydrocarbon, and polyaromatic hydrocarbon. The microbial enumeration was done for total heterotrophic bacteria and hydrocarbon utilizing bacteria. The molecular characterization of the contaminated soil was also done using the shotgun analysis. The total heterotrophic bacteria from the unpolluted and polluted soil were 1.3×10^7 and 2.1×10^2 while the hydrocarbon utilizing bacteria for unpolluted and polluted soil was 1.6×10^5 and 1.1×10^1. The total heterotrophic bacteria from the microcosms ranged from 1.75×10^8 to 2.20×10^8 for week 1 and 1.9×10^8 to 2.25×10^8 for week 2. The molecular characterization showed the presence of 102 genera from the polluted soil with the highest phylum being Proteobacteria.

Keywords: Bioremediation, Biostimulants, Natural attenuation, Poultry dropping, Cow dung, Petroleum hydrocarbon.

Introduction

Soil pollution is the contamination of the soil with pollutants, toxic chemicals, or any contaminant in such a quantity that reduces soil quality and makes it to habitable to organisms such as insects and other microbes [1]. It is the result of human interaction and activities in the ecosystem through physical, chemical, and biological scientific activities, leading to the spread of destructive or harmful substances [2]. Oil spills result from excessive oil exploration and development, resulting in spills into the environment, causing destructive and harmful activities of ecosystems, resulting in national economies [3].

The Niger Delta is a rich oil ecosystem situated in the southern part of Nigeria, which covers an area of approximately 70,000 km² and is known as African's largest wetland, also considered as a notable wetland and marine ecosystem in the world. About 95% of the crude oil produced in Nigeria is from the Niger Delta region and is said to have a great impact on the sustenance of Nigeria's economy [4]. Crude oil is a complex mixture of a vast number of individual chemical compounds [5]. The Niger Delta region experiences a high number of oil spill incidents because it is the seat of crude oil activities in Nigeria [6]. These activities that cause oil spills could be mistakes resulting from the operational processes during production, deliberate acts of sabotage during the production process, and accidents involving oil storage facilities oil tankers [7].

Another major cause of the oil spill is the disruption of pipelines in the attempt to steal oil from pipelines [8]. The disruption of the soil with crude oil leads to a loss of agricultural lands, a loss of microbial structure, ecosystem services, and source of livelihood for indigenous citizens of the Niger Deltans due to the health problems associated with exposure to oil such as respiratory damage, decreased immunity and increased cancer risk [9]. It becomes imperative to reduce, remove or completely stop these harmful activities. The ecosystem can be restored using bioremediation.

Bioremediation

Bioremediation is a process that involves the use of biological processes to return the environment to its original state [10], it is an alternative method to detoxify contaminants, and an effective means of mitigating hydrocarbon, non-chlorinated pesticides and herbicides, nitrogen compounds, and metals [11]. This alternative technique is effective, less hazardous, economically, and environmentally friendly, and versatile in the clean-up of pollutants from the environment [12]. Bioremediation achieves its purpose through biodegradation which is known to be a technique that utilizes microorganisms or their enzymes and metabolic activities to restore environmental damage to a state that is suitable for use [13]. The petroleum contaminated sites having less efficient and metabolically poor microbial populations can be remediated significantly by the addition of some of the rate-limiting nutrients or through the process of bio-stimulation [14]. Various researchers have dealt with nutrient applications as a bio-stimulant for bioremediation of petroleum polluted sites and have employed the use of agricultural wastes as nutrient supplements [15, 16]. Reported work on the comparative effect of cow dung manure on soil and leaf nutrient and yield of pepper was limited to two field trials which were conducted involving six treatments replicated three times in a randomized complete block design at Ondo, Southwest Nigeria [17]. The result showed that the addition of cow dung manure to the soil made a significant change in the growth and fruit yield parameters such as numbers of leaves and branches, plant height and girth, and the number and weight of fruits increased [17].

This work aimed to identify the best nutrient amendment for the bioremediation of the oil-contaminated agricultural soil used in this study.

2. Materials and Methods

2.1 Sample Collection

The soil samples were collected from 4°51'19.5"N 7°01'33.1"E at a depth of 0-30cm and 30-60cm and the samples were mixed to obtain a composite sample. The soil samples were artificially contaminated with crude oil and placed in earthen pots, to which the 10g of amendments were added and turned periodically to allow aeration.

2.2 Physicochemical characterization

The physicochemical parameters like total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbon were analyzed using gas chromatography. The pH was carried out using the ASTM D1293B method, electrical conductivity was done using the ASTMD1125 Method, total organic matter using the routine colorimetric method, temp was done using APHA 2005, and phosphorus using the EM Test kit 5934.

Enumeration of total heterotrophic bacteria counts

The Total Heterotrophic Bacteria (THB) populations of soil samples were determined using the spread plate method on nutrient agar [18]. Serial dilution was carried out and 0.1 ml diluent was cultured using the spread plate method.

Enumeration of hydrocarbon utilizing bacteria (HUB) counts

One gram (1 g) of soil was homogenized in distilled water, plated out on Bushnell-Haas agar modified with Agar-agar (to aid solidification) using appropriate dilutions of 10^{-5} and 10^{-6}. Hydrocarbon was supplied through the vapor phase transfer technique by placing sterile Whatman No.1 filter paper saturated with 5ml of crude oil in the inside lid of each plate kept in an inverted position and incubated at 37^{0}C for 48 hours. Bacteria growth in Bushnell-Haas agar becomes visible from 3-7days after inoculation, colonies formed were counted and expressed as colony-forming units.

3. Results and Discussion

Physicochemical Characteristics

The texture analysis of soil done using the hydrometer showed sand has a percentage of 40.3 %, clay has 20.08 % and silt 39.62 % which is a ternary classification as loam soil (Table 1).

The chemical characteristics of the pristine soil were analyzed for parameters like pH, electrical conductivity, temperature, total organic carbon, and total nitrogen (Table.2).

Table 1: Physical characterization of the soil used in the study

Soil texture parameters	Values
Moisture content (wt%)	41.97
Bulk density (g/cm3)	2.596
Water holding capacity	35.91
Sand (%)	40.3
Silt (%)	39.62
Clay (%)	20.08
Porosity (%)	49.45
Soil texture	Loam

The physicochemical characteristics of the pristine soil was analyzed at 25 ^{o}C prior to pollution. The total hydrocarbon content of the soil was less than 0.1 mg/kg indicating the absence of any hydrocarbon contamination (Table 2a); however, the polluted soil had a total petroleum hydrocarbon (TPH) of 34342 mg/kg and a polyaromatic hydrocarbon (PAH) concentration of 96.45 mg/kg (Table 2b).

Table.2a: Physicochemical Characteristics of pristine soil

Physicochemical characteristics	Value	Methods
pH @ $25^{O}C$	6.49	EPA 9045D
Conductivity	35.17	SSST EC (EC METER)
Organic matter (%)	3.01	Walkley-Black
Total Organic Carbon (%)	1.94	Walkley-Black
Cation Exchange Capacity (meq/100g)	18.64	EPA 9081
Total Nitrogen (mg/kg)	1.98	ASTME258
Total Phosphorus (mg/kg)	8.27	EPA 365-3
Total hydrocarbon content (mg/kg)	<0.01	ASTM D7066
Total Nitrogen (mg/kg)	1.90	Kjeldahl Method
Sulphate (mg/kg)	1.68	APHA 4500-SO3
Chloride (mg/kg)	3.67	APHA 4500-C1
Nitrate (mg/kg)	0.70	APHA 4500-NO3
Temp ^{O}C	25	APHA 2005

Table.2b: Hydrocarbon fractions in polluted soil.

Physicochemical characteristics	Value	Method
TPH (mg/kg)	34342	USEPA 8015C
PAH (mg/kg)	96.45	USEPA 8270D

Total Petroleum Hydrocarbon (TPH)

The TPH of the different microcosms showed a reduction in the level of hydrocarbon in the soil, the polluted soil without amendment ranged between 34,000 mg/kg and 16,000 mg/kg at weeks 2 and 4 respectively. The microcosm with cow dung amendment showed the most reduction within the four weeks this study was conducted (from 23500 mg/kg to 4950 mg/kg), the microcosm with poultry dropping reduced from 24500 mg/kg at week 2 to 16000 mg/kg at week 4, the microcosm with N.P.K reduced from 20000 mg/kg to 6500 mg/kg at weeks 2 and 4 respectively.

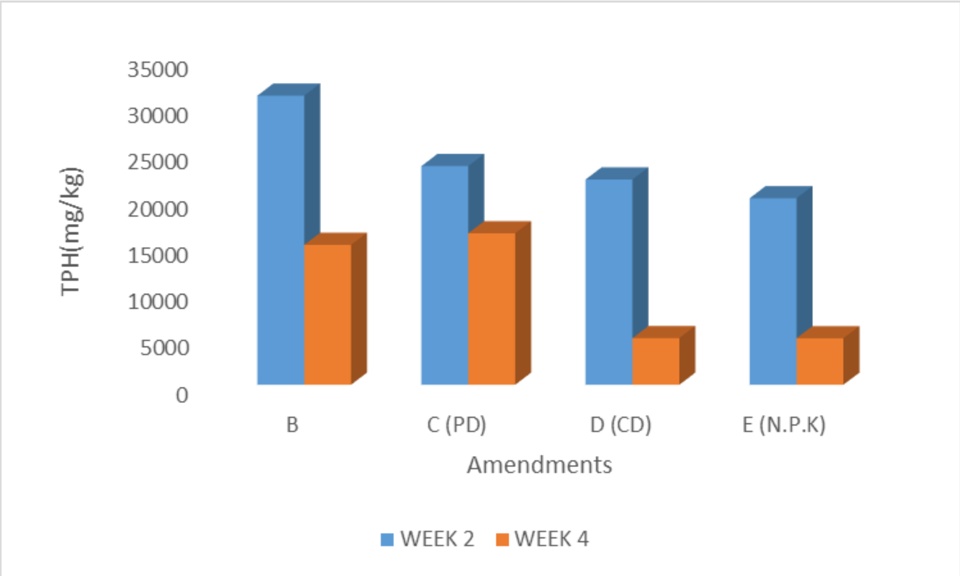

Fig 1: Hydrocarbon fractions observed in microcosms, where B is spiked soil, C(PD) is poultry-dropping treated soil, D(CD) is cow-dung treated soil, and E(NPK) treated soil.

Polyaromatic hydrocarbon (PAH)

Polyaromatic hydrocarbon fractions were effectively removed by all the microcosms with cow dung being the highest (from 96.45 mg/kg to 2 mg/kg) and the control is the least (from 96.45 mg/kg to 16 mg/kg).

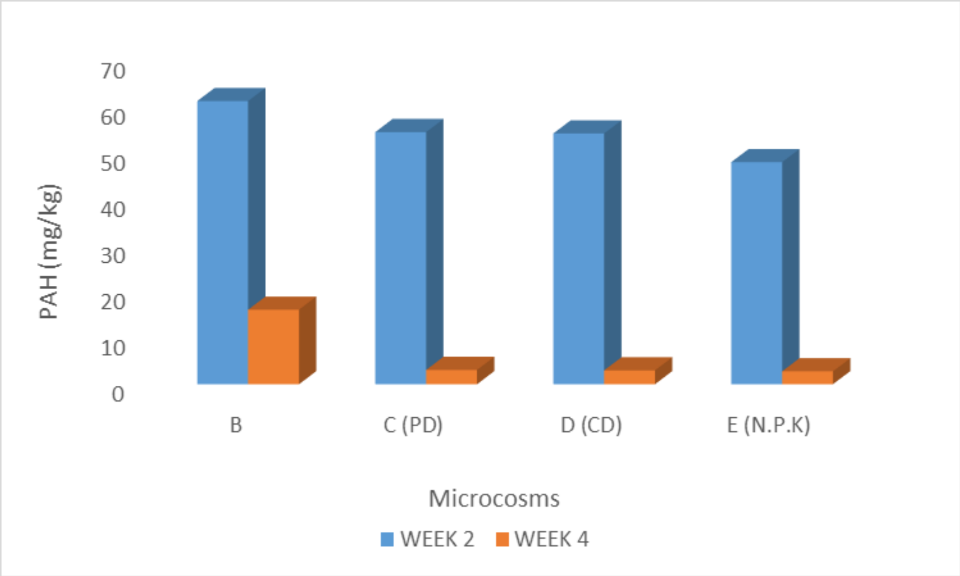

Fig 2: Polyaromatic fraction removal by microcosms at weeks 2 and 4.

Total Heterotrophic Bacteria (THB)

The treatment without amendment (B) had 1.75 X 10^8 and 1.90 x 10^8, amendment C with poultry dropping (PD) had 1.89 x 10^8 and 2.10 x10^8, the amendment with cow dung had 1.5 x $10^8$and 2.20 x 10^8, and the amendment with N.P.K had 2.20 x 10^8 and 2.25 x 10^8 for weeks 2 and 4 respectively.

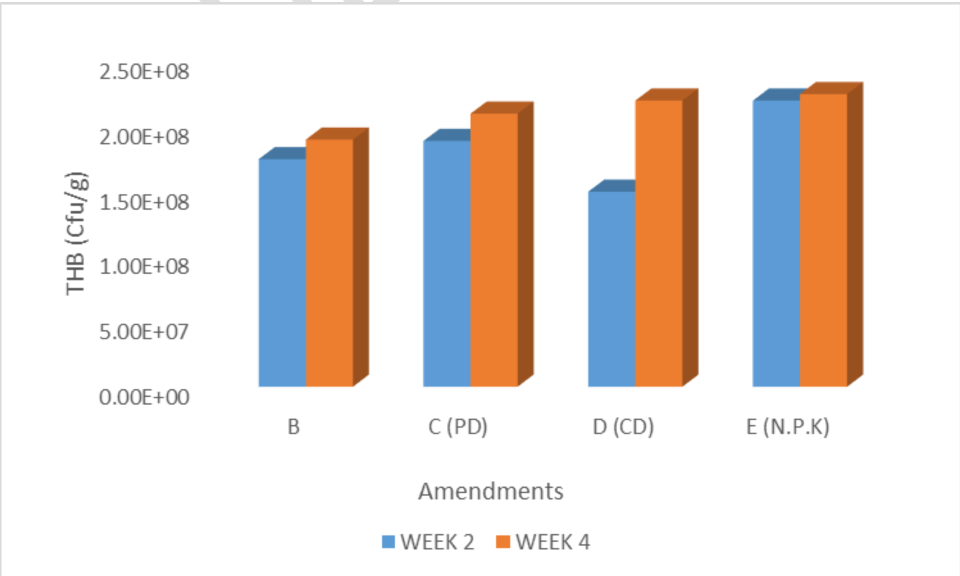

Fig 3: Total Heterotrophic Bacteria from microcosms at weeks 2 and 4 where B is spiked soil, C(PD) is poultry-dropping treated soil, D(CD) is cow-dung treated soil, and E(NPK) treated soil.

Hydrocarbon utilizing bacteria (HUB)

The hydrocarbon-degrading bacteria increased at week 4 with the cow-dung-treated soil having the highest number of degraders. The HUB count at week 4 was 2.0 x 106, 2

Fig 4: Hydrocarbon degrading bacteria from microcosms at weeks 2 and 4.

Bacterial isolates identified after biochemical characterization in this study were Pseudomonas sp, Escherichia spp, Bacillus spp, Micrococcus, and Staphylococcus.

The degradation rates of the different amendments were observed to be highest in N.P.K and the least was cow dung at week 2, however, at week 4 cow dung increased in the total heterotrophic bacterial count. The hydrocarbon utilizing bacterial counts was highest in cow dung indicating that the amendment cow dung is most effective in enhancing the degradation of hydrocarbons in the soil. This ability to degrade contaminants in the soil will encourage the proliferation of bacterial species that will enhance many ecosystems service functions.

4 Conclusion

The toxic and carcinogenic substances in crude oil-polluted soils can be removed during bioremediation via the use of nutrient amendments. During the bioremediation study, there was an increase in total heterotrophic bacteria and hydrocarbon utilizing bacteria in the microcosms showing the ability of microbes to break down the pollutants and use them as their sole source of energy. Amongst the amendment used in the study, cow dung was able to support the growth of hydrocarbon degraders thereby reducing the most contaminant making it the most effective amendment used in this study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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