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Post Remediation Assessment of Residual Hydrocarbons in Contaminated Soil in Ogoni Using Gas Chromatographic Fingerprinting Technique and Phytotoxicity Bioassay

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Abstract

Post-remediation assessment of residual total petroleum hydrocarbon (TPH) in an aged crude oil-contaminated soil (ACOCS) in Ogoni after seventy-day enhanced remediation by bio stimulation was investigated using gas chromatographic fingerprinting (GCF) technique and phytotoxicity bioassay. Seven treatments were designed and composted water hyacinth (EC), Mexican sunflower (TD) and Bermuda grass (CD) was applied as bio stimulants. Composted EC, TD or CD (2,500 g) was incorporated singly and in various combinations into 4,000g of ACOCS *in situ*. Soil treatments consisted of TPA (un-amended), TPB (amended with EC), TPC (amended with TD), and TPD (amended with CD). Others include TPE (amended with EC and TD), TPF (amended with EC and CD) and TPG (amended with EC, TD and CD). There was significant ($\rho > 0.05$) reduction in total petroleum hydrocarbon (TPH) in TPG from 93,867 to 1,002 ppm when compared to TPA which had TPH reduced from 98,673 to 79,583ppm. Gas chromatographic fingerprints of ACOCS before treatment indicated absence of *n*-alkanes within *n*-C_2 to *n*-C_8 region which was attributed to weathering processes. However, after treatment with the substrates, carbon lengths *n*-C_9 to *n*-C_{34} were significantly ($\rho > 0.05$) attenuated while those from *n*-C_{35} to *n*-C_{45} showed a decreasing tendency for enhanced attenuation thus, signifying their possible immobilization in particle pores. Seed germination index was ≥ 65%, indicating that the remediated soil is non-phytotoxic and could support plant growth.

Keywords: Post-remediation; Ogoni; Residual hydrocarbons; Bio stimulation; Phytotoxicity

Introduction

Total petroleum hydrocarbon (TPH) is a term used for any mixture of hydrocarbons that are found in crude oil. It is comprised of a very large family of several hundred chemical compounds [1,2]. Petroleum hydrocarbons are of environmental interest because they are toxic to the human system, plants and animal resources [3,4].

Yet, they pervade the environment beyond the vicinities of petroleum exploration and production activities due to storage, disposal and other handling activities during which contamination of the environment sometimes occur. Toxicity effects of total petroleum hydrocarbons in different environmental media have been studied [3-5]. The concentrations of total petroleum hydrocarbons recorded in an aged crude oil-contaminated soil in Yorla, Ogoniland have been reported [6] to be above the Department of Petroleum Resources (DPR)/Environmental Guidelines and Standards for the Petroleum Industries in Nigeria (EGASPIN) intervention limit of 5000 mg/kg [7].

Gas chromatographic fingerprinting requires using a gas chromatograph in analyzing the oil for hydrocarbon fractions in the spilled oil [2]. It is a representation of the relative concentration of compounds present in hydrocarbons. It has been demonstrated by Solomon et al. [8] that enhanced remediation of crude oil-contaminated soil using plant-based organic biomasses that is comprised of composted *Eichhornia crassipes, Tithonia diversifolia* and *Cynodon dactylon* as biostimulant could lead to a significant ($\rho > 0.05$) reduction in the residual concentration of hydrocarbons in the soil. Combinations of different bio stimulants followed by tilling gave a drastic reduction of TPH content from 93,867 ppm obtained on day 0 to 1,002 ppm (99% loss) after 70-day. The data is in agreement with the DPR/EGASPIN target value of 50 mg/kg [7].

Phytotechnology is a set of technologies using plants (roots, shoots, tissues, and leaves) to remove, transfer, stabilize, or destroy contaminants in media. Phytoremediation is an *in situ* technique that uses plants and/or its parts to restore contaminated media [9,10]. It applies to all biological, chemical, and physical processes that are influenced by plants that aid the cleanup of contaminated media. Plants materials aid degradation of organic pollutants directly or indirectly by supporting microbial growth [11-13].

Their roots are responsible for absorption and accumulation of hydrocarbon contaminants in soil [14]. Crude oil concentration in crude oil polluted soil affected plant growth and particularly the root lengths more than other parts [15]. The bioassay is useful in the evaluation of:

(i) The toxic effects of crude oil pollutants on plants and

(ii) The changes in the soil effect on plants after remediation measures [15,16].

The research, therefore, was aimed at post-remediation assessment of the residual hydrocarbons present in an aged crude oil-contaminated soil after 70-days remediation by bio stimulation using both gas chromatographic fingerprinting technique and phytotoxicity bioassay.

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Materials and Methods

Sample collection

Five grams (5 g) each of composite crude oil-contaminated soil samples was weighed form the seven (7) bio-treatment plots which included the control set-up into clean, dry beakers. Whole green plant samples of water hyacinth, Mexican sunflower and Bermuda grass were collected, composted in container and allowed to decay for two weeks.

Bio stimulation treatment plots

Seven bio-treatment plots were set-up with an area of 50 m × 50 m marked out on each of the plot site. Seven treatment plots (TPA, TPB, TPC, TPD, TPE, TPF, and TPG) each containing 4000 g of ACOCS were used for this experiment. TPA contained ACOCS only and without amendment.

The TPA served as control and served as control to simulate natural attenuation processes [11,13]. Furthermore, TPB, TPC and TPD set-ups were stimulated singly with 2500 g of composted water hyacinth (Plot B), Mexican sunflower (Plot C) and Bermuda grass (Plot D) respectively. TPE, TPF and TPG were supplemented in with 2500 g of composted plant biomass of water hyacinth and Mexican sunflower (Plot E).

Plot F contained water hyacinth and Bermuda grass while Plot F has water hyacinth, Mexican sunflower and Bermuda grass combined. The treatments were monitored for hydrocarbon biodegradation as reported by Solomon et al. [11,13].

Crude oil extraction from soil and gas chromatographic analysis

Five grams (5 g) of homogenized soil samples were accurately weighed into clean, dry beakers. The weighed samples were extracted with 10 ml of hexane respectively and passed through a filter paper [17-19].

The extract (hydrocarbon/hexane mixture), now ready for gas chromatography, was injected into a Varian model 3400 gas chromatograph (GC) with the following operational conditions; flow rate (H_2 30 ml/min, air 300 ml min and N_2 30 ml/min); injection temperature (50°C), detector temperature (320°C); recorders' voltage (IMV); and chart speed 1 cm/min. For interpretation of results, the gas chromatogram recorder was interfaced to a Hewlett Parker (hp) Computer (6207AA Software, Kaya XA PIT/350 W/48 megabytes CD-ROM). The chromatograms were quantified with respect to internal standards [20-22].

Phytotoxicity bioassay

Seed germination bioassay of remediated soil was carried out using lettuce plant seed (*L. sativum*). Ten grams (10 g) of remediated aged crude oil-contaminated soil was collected from the 7 treatment plots and suspended in 100 ml of distilled water in transparent test plates. The mixture was vigorously shaken for 30 min and the supernatants collected for seed germination bioassay [23,24].

Microcosm was set set-up in "transparent test plates" and incubated vertically for 72 h at 25 ± 2°C in the dark to allow the roots of the germinated seeds to be seen. Percentage germination index (GI%) of the plant seed was calculated from the number of germinated seeds and root length elongation of 5 mm values in the TPA as well as in the bioremediated treatment plots. The germination index (GI%) was evaluated using the expression:

$$GI\% = \frac{Gs \times Ls}{Gc \times Lc} \times 100$$

Where,

Gs is the number of germinated seeds in the bioremediated soil,

Gc is the number of germinated seeds in un-amended control soil,

Ls is the average of root lengths in the bioremediated treatment soil,

Lc is the average of root lengths in the un-amended control treatment soil.

Statistical analysis of data

Data obtained were subjected to statistical analysis to determine the significant difference among the data obtained using one-way analysis of variance (ANOVA). A value of $p>0.05$ was considered significant while $p>0.05$ was considered not significant.

Results and Discussion

The concentrations of residual fractions of total petroleum hydrocarbon and polyaromatic aromatic hydrocarbons in the soil after post- remediation study period are shown in Figures 1 and 2. Data obtained indicated that carbon lengths between *n*-C_1 – *n*-C_8 were absent.

The absence of low molecular weight hydrocarbons in the treated sample could be attributed to natural attenuation processes of weathering [25-27]. Data showed the distribution of paraffins ranging

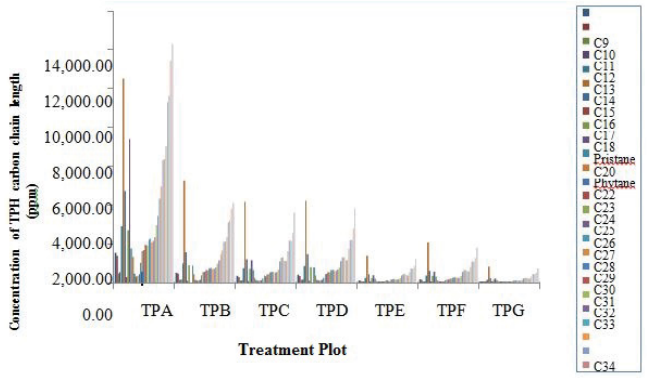

Figure 1: Concentrations of residual fractions of total petroleum hydrocarbon (TPH) in crude oil-contaminated soil during the study.

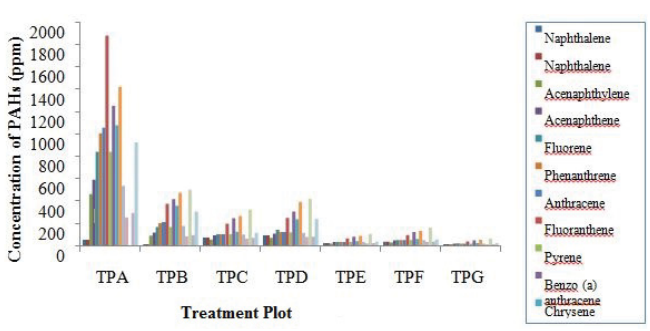

Figure 2: Concentrations of residual fractions of polycyclic aromatic hydrocarbons (PAHs) in the treatment during the study.

from n-C_9 to n-C_{23} with the fractions n-C_9, n-C_{12}, n-C_{13}, n-C_{14}, n-C_{15}, n-C_{16}, n-C_{18}, pristane and n-C_{23} being relatively high in concentrations.

Carbon lengths between n-C_9 to n-C_{34} were significantly ($p>0.05$) attenuated in the treatment plots, thus indicating that the residual crude oil in the soil environment was utilized by autochthonous microbes as sole source of carbon thereby resulting to its biodegradation [28,29].

The samples also showed a distribution pattern of odd carbon-numbered alkanes being much abundant than even-numbered alkanes in the lower alkane range. Carbon fractions between n-C_{35} to n-C_{45} were not significantly ($p<0.05$) attenuated at the end of the study period, but was found to have exhibited a decreasing tendency for enhanced attenuation, thus, indicating the need for continuous nutrient supplementation, nutrient combination and possible extension of study duration.

The PAHs fractions was found to have been reduced in all treatment plots when compare to the control and this could be due to a combination of nutrient amendments added in the plot. Careful selection and combinations of different organic amendments could lead to a drastic attenuation of the heavy hydrocarbon fractions during enhanced remediation of crude oil-contaminated soil. The attenuation of these different hydrocarbon components as depicted by the trend in the disappearance of low-molecular-weight hydrocarbons from the various bio-treatment plots could probably be due to their preferential utilization by the autochthonous microorganisms in the contaminated soil environment [29,30].

Gas chromatographic tracing of treatment plots indicated considerable residual hydrocarbon in the different attenuation of the various carbon fractions in the amended treatment when compared to the control, suggesting enhanced biodegradation of the crude oil in the soil.

Data obtained showed the pristane/phytane ratios of 3.80, 5.69, 4.83, 5.30, 4.82, 4.83 and 24.14 for TPA through TPG respectively and this depicted both artificial and petrogenic input. A pristane/phytane ratio of 5.70 has been reported by Osuji et al. [2] to be of plant/terrestrial source input and a possible toxic depositional environment. The n-C_{17}/pristane ratio of 1.53 was obtained for TPA while treatment plot B (TPB through TPG) had ratios that ranged from 1.02 to 1.11. Furthermore, n-C_{18}/phytane had ratios of 15.9 for TPA and TPB, 8.57, 9.39, 8.57 and 8.58 for TPC, TPD, TPF and ratio of 42.9 for TPG.

Figure 3 shows the structural characteristics of the various PAHs compounds in the soil environment. Members of the polyaromatic hydrocarbons (PAHs) include the following: Naphthalene, Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo (*a*) anthracene, Chrysene, Benzo (*b*) fluoranthene, Benzo (*k*) fluoranthene, Benzo (*a*) Pyrene, Indo 1,2,3 cd Pyrene, Dibenzo (*a,b*) anthracene and Benzo (*ghi*) perylene [30,31].

The abundance of high-molecular-weight PAHs suggest that these hydrocarbon components were released from petrogenic source (i.e., from petroleum). The phenanthrene/ anthracene ratios obtained ranged from 0.95 to 1.02, while the fluorathene/pyrene ratios ranged from 1.7 8 to 1.99 for the various treatments. Our results corroborated the report of Osuji et al. [2] who had similar phenanthrene/ anthracene and fluorathene/pyrene ratios of 0.95 and 2.23 respectively during their study on attenuation of petroleum hydrocarbons by weathering. The benzo (a) anthracene to chrysene ratios obtained in the various treatment plots ranged from 1.16 to 1.96 thus, confirming the non-petrogenic origin of the petroleum hydrocarbon in the soil.

Advances in the development of gas chromatographic fingerprinting

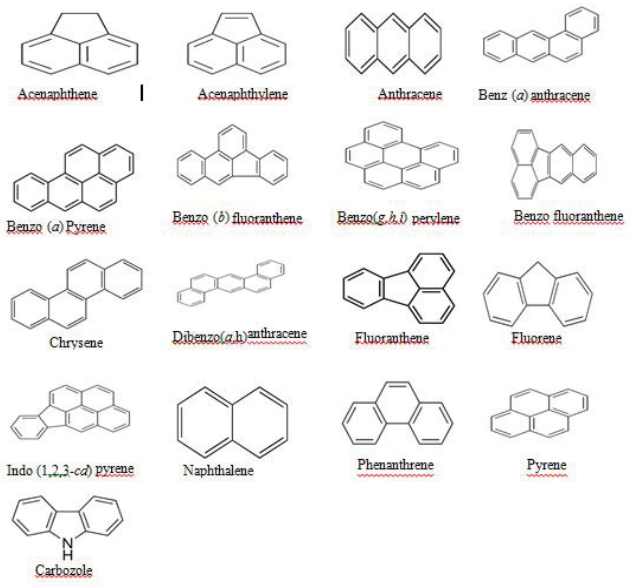

Figure 3: Structural characteristics of PAHs compound present in the soil environment.

(GCF) techniques will continue as analytical and statistical methods are developed. Hence it is believed that in the nearest future, these developments will further make gas GCF technique, a veritable tool for oil spill source identification and differentiation.

The trends in PAHs peak height attenuation could be attributed to the metabolic activities of PAH-degrading microbes which mediate the changing PAHs conditions in the soil as smaller molecules are being used to build larger ones and complex molecules broken down into smaller ones [20,28,30,32].

Data obtained indicated that there was no significant ($p<0.05$) reduction in PAHs concentration TPA but reduced significantly ($p>0.05$) in TPB through TPG following nutrient supplementation. Figures 4–6 show the GCF peaks heights of TPH and PAHs in TPA, TPB, TPC, TPD, TPE, TPF and TPG after the 70-day study.

This suggested that the crude oil was not only slightly weathered but biodegraded by hydrocarbon utilizing microbes, as corroborated by the number of peaks in the total petroleum hydrocarbon fingerprints. The GCF data obtained show various carbon lengths and PAHs components with decreasing peaks values (Figures 4-6) indicating presence of hydrocarbons from petroleum origin. Phytotoxicity bioassay has been described as an intoxication of living plants by substances in the growth medium, when these substances are accumulated in plant tissue [32,33]. The germination index (GI) of *L. sativum* obtained in the bio remediated soil followed a similar order as residual total hydrocarbon reduction in the treated plots. The phytotoxic capacity of the remediated soil after 70-day enhanced remediation study period via bio stimulation with plan-based manures indicated a gradual disappearance of phytotoxicity in the various bio-treatment plots (TPB through TPG) thus, indicating a significant ($p>0.05$) reduction of TPH in the TPG-amended treatment plot.

The pristine soil used as negative control, showed no toxicity to seed germination and is thought to contain only biogenic hydrocarbons. The seeds did not germinate at the onset of the test on 0d in all treatments

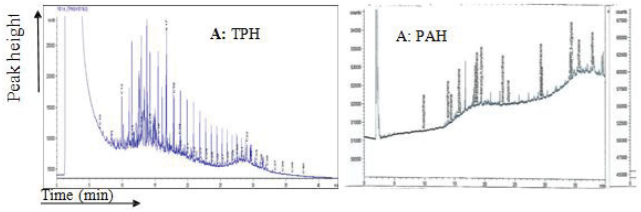

Figure 4: GCF of residual TPH and PAHs components in TPA after 70D remediation study.

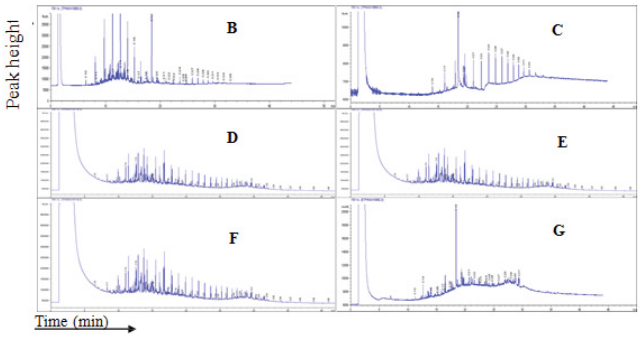

Figure 5: GCF of residual TPH in treatment plots B, C, D, E, F and G after 70D remediation.

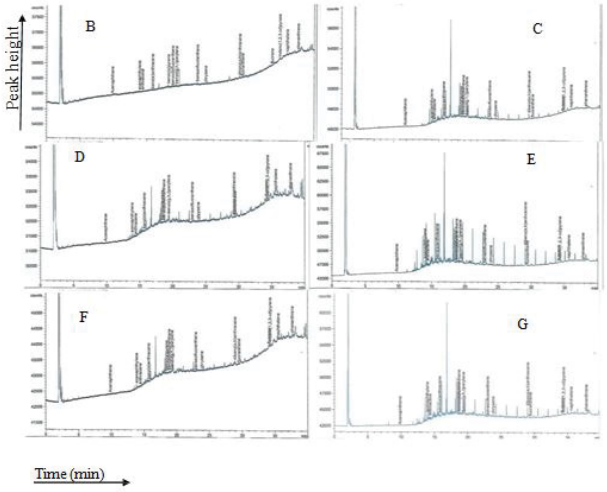

Figure 6: GCF of residual PAHs in treatment plots B, C, D, E, F and G after 70D remediation

but after 70-days of phytotoxicity testing, all the seeds germinated maximally in the treated soil, indicating no toxic effect of the residual crude oil in the soil after remediation [34].

Figure 7 indicated GI of 0.11% in the un-amended TPA, 65% in TPB-amended with water hyacinth, 76% in TPC amended with Mexican sunflower and 71% in TPD-amended with Bermuda grass respectively. Whereas the GI of TPD amended with water hyacinth and Mexican sunflower was 91% while TPE-amended with water hyacinth and Bermuda grass recorded 83% while TPG-amended with all three nutrients of water hyacinth, Mexican sunflower and Bermuda grass had

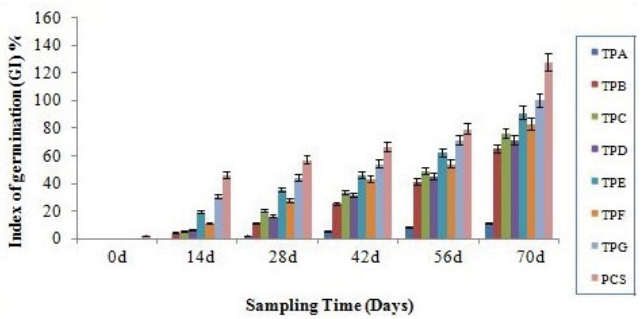

Figure 7: Index of germination of Lettuce plant (*Lepidium sativum*) seeds in the different treatment plots.

germination index (GI) of 100%, thus indicating no toxic effect and bio-safety of the crude oil pollutants on plant growth.

A GI less than 40% has been proposed to indicate a high toxicity while a value ranging from 40% to 60% indicates moderate toxicity (with delay in the seed germination) and values higher than 60% indicates the absence of any negative effect [35-38]. The results obtained indicated that TPA had GI of 0.11%, giving high toxicity effect and could be probably due to the absence of nutrient amendments in the control set-up.

Conclusion

Advances in gas chromatographic fingerprinting technique allowed for detailed qualitative and quantitative characterization of spilled petroleum hydrocarbon and subsequent source identification. Phytotoxicity endpoints are useful indicators for the assessment of the quality of an environmental medium as a habitat for micro-fauna and flora.

It is generally useful in checking for toxicant concentrations that are bioavailable for adapted microbial species and other exogenous organisms in the crude oil-contaminated soil environment. The bio remediated soil could be considered non-phytotoxic and ecologically safe (without toxic impacts on seed germination) since the germination index of *L. sativum* in treated plot ranged between 65 and 100%. Data obtained confirmed the remediated soil's recovery potential and bio-safety level to ecotype. Plant-based organic manures used in this study are potent bio stimulating agents.

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