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Original Paper

Uptake and bioaccumulation of diverse hydrocarbon compounds by selected food plants artificially exposed to bioremediated crude oil-contaminated soils

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Assessment of the uptake and bioaccumulation of diverse hydrocarbon compounds within internal tissues by selected food plants artificially exposed to bioremediated crude oil-contaminated soils was carried out. Three bioremediated crude-oil contaminated soils of different fallow ages (6-, 12-, and 18- months after certified remediation protocols) and an uncontaminated soil were collected and designated as 6m-AB, 12m-AB, 18m-AB and control respectively. Total petroleum hydrocarbons (TPH) and intermediate metabolites of degradation in soil samples were determined in the dry and wet seasons using Gas Chromatography – Mass Spectrophotometer. *Telfairia occidentalis*, *Zea mays*, *Cucumis sativus*, and *Abelmoschus esculentus* were used to assess safety of crops grown on test soils by monitoring the bioaccumulation of chemical residues in their tissues. Baseline TPH contents in various soil samples were 161.25 mg Kg^{-1} (6m-AB), 51.72 mg Kg^{-1} (12m-AB), 91.50 mg Kg^{-1} (18m-AB) and below detectable level in the control soil. A myriad of organic compounds emanating from degradation of petroleum compounds and including toxic and carcinogenic metabolic intermediates like trifluoromethyltrimethylsilane, phthalate esters and halogenated aliphatics were detected in bioremediated soil and also in tissues of the plants grown on the bioremediated soils. Higher bioconcentration factors for accumulated organic compounds were obtained during the wet season for all plants with *Telfairia occidentalis* having the highest bioconcentration factor in both wet and dry seasons. Results obtained provide evidence of contaminant transfer from these bioremediated soils to plant tissues and suggest the need for adequate evaluation of chemical residues in remediated soils before utilizing such sites for farming to ensure safe crop production.

Keywords: crude oil-contaminated soil, bioremediation, bioconcentration, plants, TPH

1 Introduction

The ever-growing demand for petroleum products by emerging economies has exacerbated the issue of crude oil contamination in the environment, which is of great concern. The total oil consumption in 2009 was 82,769,370.4 bbl day^{-1} out of which Nigeria's production was 2,520,000 bbl day^{-1} (CIA, 2012). In order to meet this demand, there has been increased exploration of crude oil reservoirs leading to a surge in oil spills arising from activities like extraction, processing, transportation and storage of petroleum and petroleum products from the oil industry. Soil pollution occurs when hydrocarbons present in crude oil are released/spilled into the environment via anthropogenic activities. Kostka et al. (2011) had reported that hydrocarbon contamination has immensely affected natural resources thus, exerting negative impacts on the natural environment and economic growth. Environmental pollution caused by crude oil spills goes beyond what can be sensually perceived as there are dire effects that threaten biodiversity, ecosystem and environmental balance owing to leaching, extension and bioaccumulation of

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contaminants from soil with possible effects on living organisms (Ortínez et al., 2003). Significant changes in chemical and physical characteristics of the soil, retarded plant growth and development as well as limited microbial growth can result from exposure to crude oil (Vázquez-Luna, 2012).

Several authors have reported the use of bioremediation as an effective method in eliminating different organic pollutants from soil, thereby reducing their toxicity (Vidali, 2001). Unfortunately, decrease in the degree of crude oil contamination does not always translate to decrease in soil toxicity. Soil toxicity could be worsened by the emergence of intermediate metabolites, partial degradation and persistence of heavy metals (Phillips et al., 2000). Hence, there are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound which may result in poor crop yield when such bioremediated sites are used for plant cultivation. It has been established that contaminants can cause several alterations in plant growth and development (Khan et al., 2008) albeit, the effect metabolic intermediates and residual hydrocarbons from bioremediation have on plant is still not known. Moreover, some of these intermediate metabolites, residual petroleum hydrocarbons and heavy metals are carcinogenic and may exacerbate incidences of cancer in humans if they are bioacccumulated in crops and eventually consumed (Khan et al., 2008). Mmom and Deekor (2010) pointed out that environments contaminated with crude oil threaten water supply, fishes, farmlands, as well as livelihood of people that depend on farming and fishing. In addition, Petts et al. (2000) stated that the effect of industrial activities from history, can have deleterious effect on man as well as the environment, depreciate the value of land, and also limit the safe re-use of lands. Tinsley and Farewell (2015) pointed out that land contamination has changed several useful lands into waste lands.

Since decrease in total petroleum hydrocarbons, only, cannot provide an overview of the complex process of bioremediation, there is need to investigate the presence of metabolic intermediates arising from the degradation of petroleum hydrocarbons. Chemical analyses in terms of total petroleum hydrocarbon content and polycyclic aromatic hydrocarbon content of soil give information (e.g. bioavailability of contaminant) to help predict the success of bioremediation process however, generation of intermediate metabolites which persists after the bioremediation process may present even a bigger toxicity problem. Therefore, an understanding of the array of residual contaminants and metabolic intermediates left behind in soil after a bioremediation intervention is important to determine the recovery extent of contaminated soil and the total wellbeing status of the ecosystem. Thus, after a certified bioremediation process, evaluating the presence of these compounds (metabolic intermediates and residual hydrocarbons) and their effect irrespective of their minute concentrations in soil is vital. The combination of data from remediation potential and chemical analysis is required to correctly evaluate the ecological risk present in bioremediated soils. A successful bioremediation strategy ensures safe and healthy food crop production as well as environmental sustainability (Alburquerque et al., 2011). However, there is dearth of information on the performance of plants grown on bioremediated soil in Nigeria.

Hence, this study used plant bioassay to determine the effects of residual contaminants and intermediate metabolites in bioremediated crude oil-contaminated soil and their implications for safe crop production and food safety.

2 Materials and methods

2.1 Sample collection

Soil samples were collected for this study from bioremediated crude oil-contaminated sites in Ogoni, Rivers State in June, 2018. The sampling locations are presented in Figure 1. Selection of the bioremediated sites was based on their ages (6 months, 12 months, and 18 months) following remediation certification. A control soil sample was collected from an uncontaminated site which had not been disturbed. The obtained soil samples comprising of the bioremediated and uncontaminated (control) soil samples were sandy loam in texture. The soil samples were also similar in terms of soil type as they consisted of young sedimentary soil (histosols) probably derived from recent alluviation (Onyeike et al., 2002). A Dutch soil auger was used to obtain surface soil (0–30 cm depth) from each of the selected bioremediated sites and control site. Forty (40) soil samples were collected from all the sampling locations to make four composite samples. The sample size (number of soil samples collected) for each composite sample, at individual sites, was ten. Composite sampling was employed at each of the four locations to have a good representation of each sampling site. Prior to mixing to make the composite samples, rocks and other particles in the soil samples were eliminated. The composited samples were kept in clean, sterile polythene bags for storage and thereafter, transported to the laboratory for chemical analyses in an ice chest maintained at a low temperature (ca 4 °C).

2.2 Determination of total petroleum hydrocarbons

Total petroleum hydrocarbons present in the soil samples and plant tissues were determined using the Varian

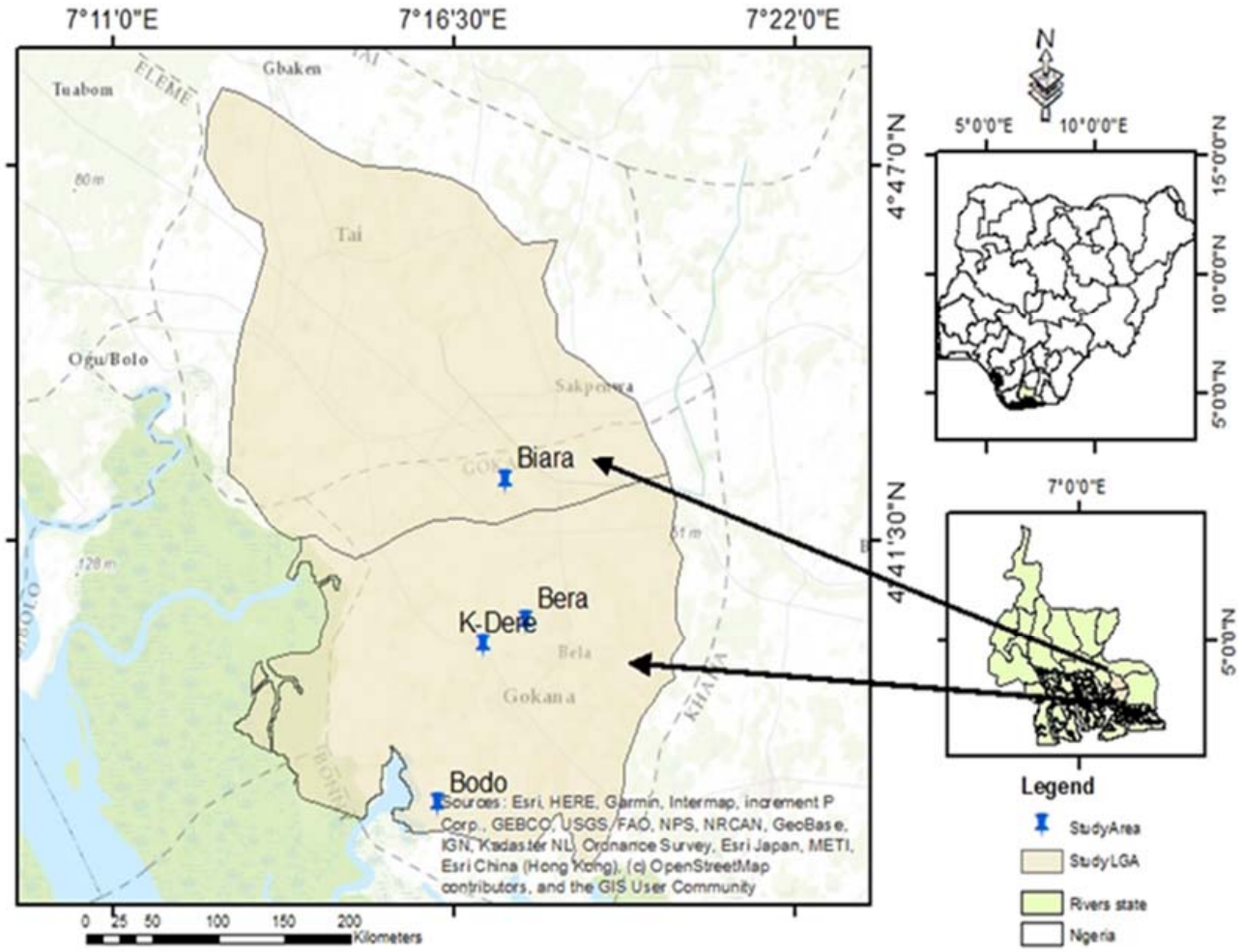

Figure 1 Map of locations in Ogoni sampled in this study

CP 3800 gas chromatography (GC) in line with ASTM D4657, EPA 1625 and USEPA 8270B (US EPA, 1988) and as previously described by Jason-Ogugbue et al. (2019)

2.3 Determination of aromatic hydrocarbons and metabolic intermediates

Aromatic hydrocarbons and metabolic intermediates present in soil samples and plant tissues were determined using the Agilent Technologies 6890 Gas Chromatography – Mass Spectrophotometer (GC-MS) as previously described by Jason-Ogugbue et al. (2019).

2.4 Assessment of food safety

The seeds of the test plants were subjected to pre-germination test using Petri dishes and tray pan (for *Telfairia occidentalis*) to determine the viability of the seeds before the actual experiment as previously described (Jason-Ogugbue et al., 2019) and they passed with more than 70% successful germination. Thereafter, well perforated plastic containers of 11 × 12 cm containing 21 kg of each soil sample were used for planting. The seeds of *Telfairia occidentalis* were extracted from the pod and the fleshy substance coating the seeds were removed. Thereafter, the seeds were rinsed and allowed to dry for 2 days before planting 6 seeds per bucket. The seeds were planted in such a way that the exposed part of the seeds faced upwards in the soil. All the seeds were placed just about 1 cm deep in each soil type. For *Zea mays*, 6 seeds were planted per bucket for each of the different soil types and were thinned to 2 seedlings one week after germination in order to obtain a 2-plant density per bucket. Ten (10) seeds of *Abelmoschus esculentus* and *Cucumis sativus* were also planted in each soil but were later thinned to 2 seedlings after one week following germination to obtain a 2-plant density per bucket.

Subsequently, the safety of crops cultivated on the bioremediated crude oil-contaminated soils was determined by evaluating the bioaccumulation trends of pollutants (TPH and metabolic intermediates) in *Telfairia occidentalis*, *Zea mays*, *Abelmoschus esculentus* and *Cucumis sativus* plants grown on bioremediated soil and

in the uncontaminated soil. Accumulation of the TPH and intermediate metabolites in vascular and ground tissues of stem and leaf of plants and the decimation of these pollutants in soil were assessed at the end of dry and wet seasons' study.

The accumulation potentials of the test plants were examined using the bioconcentration factor. The bioconcentration factor was computed using the following formula:

$$BCF = C_{biota}/C_{soil} \quad (1)$$

where:
C_{biota} – TPH concentrations per unit weight of plant tissue per unit weight of bioremediated soil; C_{soil} – TPH concentration per unit weight of bioremediated soil

2.5 Statistical Analysis

Results obtained were subjected to statistical analysis (One-way ANOVA) using the SAS software.

3 Results and discussion

Prior to the study, the three bioremediated soil samples (6m-AB, 12m-AB and 18m-AB) obtained from crude oil spill sites had been certified bioremediated 6 months, 12 months and 18 months respectively by regulatory agencies following an intervention by reclamation outfits. Results of TPH concentrations determined before and after plant cultivation in the dry and wet seasons are as presented in Figure 2.

The TPH content of the bioremediated crude oil-contaminated soils ranged from 51.72–161.25 mg kg^{-1} at the onset of the dry season study and decreased to a range of 74.5–133.23 mg kg^{-1} by the end of the dry season study. In both instances, 12m-AB soil sample had the least TPH concentration in soil while 6m-AB soil sample had the highest TPH content (Figure 2). Petroleum hydrocarbons were not detected in the uncontaminated soil (control). Generally, based on GC-MS analysis, C_{12}–C_{36} aliphatics remained in the bioremediated crude oil-contaminated soils after remediation as at the time the samples were obtained, whereas the low molecular weight short chain alkanes (C_2–C_{11}) and the high molecular weight long chain alkanes (mainly C_{34}–C_{40}) were absent indicating their preferential dissipation in the soils during remediation. The absence of C_2–C_{11} low molecular weight aliphatics in the bioremediated soils could be attributed to their volatility, solubility and high biodegradability potentials.

The release of crude oil into an environment is accompanied by alterations in its composition as a result of natural attenuation arising from influence of physical factors (dissolution, evaporation, degradation,

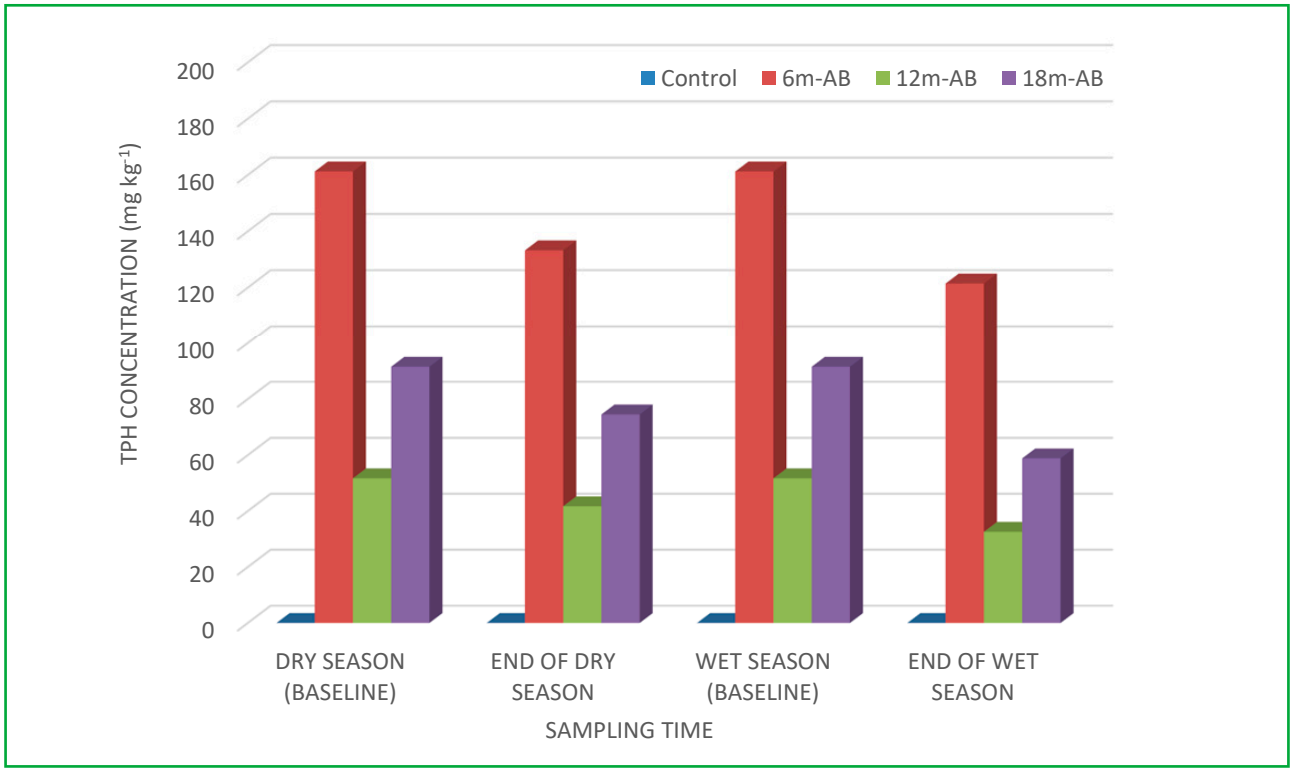

Figure 2 Changes in total petroleum hydrocarbons (TPH) concentration with time during the dry and wet seasons in various bioremediated crude oil-contaminated soil samples obtained from Ogoni

photooxidation, water – oil emulsification) and biodegradation activities (Shirani et al., 2012). It has been reported that a few days after oil spillage, the rate of loss of hydrocarbons in soil via evaporation reaches 5–10% for heavy oils, 40% for crude oil products and 70% for volatile fractions (Lee et al., 2003).

In addition, the dual property of low solubilization and slow dissolution of high-molecular-weight hydrocarbons render their uptake by microorganisms more difficult when compared to low-molecular-weight hydrocarbons which are more water-soluble. Since the biodegradation rate of hydrocarbons depends on their uptake, the high molecular weight hydrocarbons are degraded at a slower rate compared to the lower molecular weight hydrocarbons following the studies of Miller and Bartha (1989). A sufficient mass-transfer to the cell for direct uptake can still be obtained for low-molecular-weight alkanes based on their solubility however, adhesion to hydrocarbon droplets or surfactant-facilitated transfer-to-cell process may be required for uptake of medium- and long-chain-length alkanes by micro-organisms (Rojo, 2009).

Moreover, the low molecular weight hydrocarbons are easily metabolized by most hydrocarbon-utilizing microorganisms which could be the reason for their non-detection in bioremediated soils. However, degradation of medium and high molecular weight aliphatic hydrocarbons up to C_{44} also occurs in soil, albeit at a slower rate (Abbasian, 2015). The biodegradation rate mainly depends on the nature of oil, the quantity of spilled crude oil, physical factors (such as temperature, pH and salinity) and the accessibility of water, nutrients and oxygen (Collier et al., 2012). Generally, in aged crude oil-contaminated soils, branched and shorter straight chain *n*-alkanes are almost exhausted and the amount of polyaromatic hydrocarbons is also much dissipated (Yang et al., 2012). In all three bioremediated soils studies, C_{22}–C_{24} were the most abundant alkane species amongst the aliphatic hydrocarbon fractions that remained in the soil after the long post-intervention fallow period.

TPH content of 6m-AB soil sample at the onset and end of the wet season study were 161.24 mg kg^{-1} and 121.46 mg kg^{-1} respectively, whereas in 12m-AB soil, the TPH content depressed from 51.72 mg kg^{-1} at the onset of study to 32.59 mg kg^{-1} by the end (Figure 2). There were significant differences (p <0.05) in TPH contents of the bioremediated soils obtained during different seasons and from various locations in Ogoni. There was no trace of residual TPH in the uncontaminated soil sample in both seasons. The TPH loads in the bioremediated soils exceeded the recommended TPH target value of 50 mg kg^{-1} but were below the intervention level of 5,000 mg kg^{-1} as depicted in the Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (DPR, 2002). The higher-than-target residual TPH contents obtained for these bioremediated sites suggest that the soils still require mitigation measures to ensure the total restoration of the ecosystem. Criticism of the remediation procedures mainly deployed in Ogoni has been made by the United Nations Environmental Programme (UNEP). In their report (UNEP, 2011), it was stated that the remediation of crude oil-contaminated sites is mostly conducted by contractors who are not adequately instructed to utilize state-of-the-art techniques and have often been ill-supervised.

Remediation by Natural Attenuation (RENA), a form of land-farming involving the excavation and/or aeration of contaminated soil and eventual hydrocarbon breakdown by microbes (bioremediation) within the soil under atmospheric conditions, and sometimes enhanced by nutrients (bio-stimulation), is one of the most frequent remediation techniques applied by contractors in Ogoni. This remediation protocol, in many cases, has performed short of expectations and has raised concerns because of the additional contamination hazards for the locals posed by insufficiently degraded/residual hydrocarbons in backfilled material (UNEP, 2011; Thiergärtner and Holtzmann, 2014). In that report, EGASPIN was chastised for not stipulating separate values for triggering and closing out remedial interventions; a loophole that allowed negligible cleanup by contractors to be accepted by regulatory agencies (UNEP, 2011). The stipulated high TPH intervention value of 5,000 mg kg^{-1}, and the dearth of limits on individual PAHs especially benzo(a)pyrene also attracted the criticism of the guidelines (IUCN, 2013). TPH are considered acutely toxic (Heitkamp et al., 1988) and consists of compounds capable of bioconcentration and bioaccumulation in food chains (McElroy et al., 1989). Some associated compounds, such as benzene (IARC, 2000) and benzo[a]pyrene are known mutagens and carcinogens (Mortelmans et al., 1986).

The relatively higher TPH load obtained for 6m-AB may be due to its post-demobilization age status (6 months after intervention) as compared to the bioremediated crude-oil contaminated soil samples collected from other sites that had been left fallow for 12 months (12m-AB) and 18 months (18m-AB) following intervention. During the period of site fallowing, biodegradation of residual petroleum hydrocarbons is sustained by surviving microbes as long as prevailing environmental conditions remain favorable for their growth and degradation activities even after site demobilization. Stimulated or not, these native microbes capable of petroleum hydrocarbon degradation may still have an important effect on remediation, especially if site being treated was exposed to biogenic or petroleum hydrocarbons prior

to the current contamination (Bento et al., 2005). The activities of these microbes during the fallow period may be the reason for the lower residual TPH concentrations obtained for 12m-AB and 18m-AB bioremediated soils. It has been reported that biodegradation of target compounds by communities of native microorganisms is the primary mechanism for contaminant attenuation (Declercq et al., 2012) and that degradation of TPH may still occur naturally by indigenous microbes after 30 months of contamination with a 77% degradation rate (Rhykerd et al., 1999). However, it is pertinent to state that petroleum hydrocarbons may remain a pollution quagmire for years when prevailing conditions/factors (such as temperature, sunlight, oxygen, nutrients, microbial communities and type of oil spilled) are less favorable for weathering and degradation to occur (Lindén and Pålsson, 2013). The latter being why this study endeavored to determine the transfer pathways of these toxic and persistent hydrocarbons via plants presenting a potential exposure to humans as a valid starting point to protecting the local populace.

The concentrations of TPH accumulated in tissues of plants grown on the bioremediated crude oil-contaminated soils samples during this study in the dry and wet seasons are as presented in Figs 3–6. The bioconcentration factor (BCF) values of TPH in tissues of plants are also shown in Figs 7–8. When compared to other plants grown on all three bioremediated soils, *Telfairia occidentalis* grown on 12m-AB soil accumulated the highest concentration of TPH in its tissues during the dry season study, whereas the least TPH concentration was obtained in tissues of *Zea mays* grown on same 12m-AB soil. Likewise, tissues of *Telfairia occidentalis* accumulated the highest amount of TPH in 6m-AB and 12m-AB soils during the wet season study, whereas in 18m-AB soil, the highest concentration of TPH was found in *Abelmoschus esculentus* grown on that soil. Generally, higher bioconcentration factors were obtained during the wet season when compared to the dry season data (p <0.05) for all plants grown on various bioremediated soils. Significant differences (p <0.05) in bioconcentration factor of TPH in tissues of the different plants were also obtained. Adhesion of the hydrocarbons (which are generally water insoluble) to the roots due to pressure arising from inundation of soil pores with water during the rainy season may have facilitated their uptake and active translocation to the upper parts of the plants via the vascular system. In addition, dissolution of the water-soluble lighter hydrocarbon fractions in soil water during the wet season may have rendered them more available for plant uptake just as sorption to soil particles during the dry season may have made them unavailable to the roots. Tissues of various plants grown on uncontaminated soil in both seasons did not show any detectable TPH content. For most plants used in this study, the bioconcentration factor was more than 1.0 especially during the wet season indicative of efficient uptake of petroleum hydrocarbons via the test plants' root network (Lotfinasabasl et al., 2013)

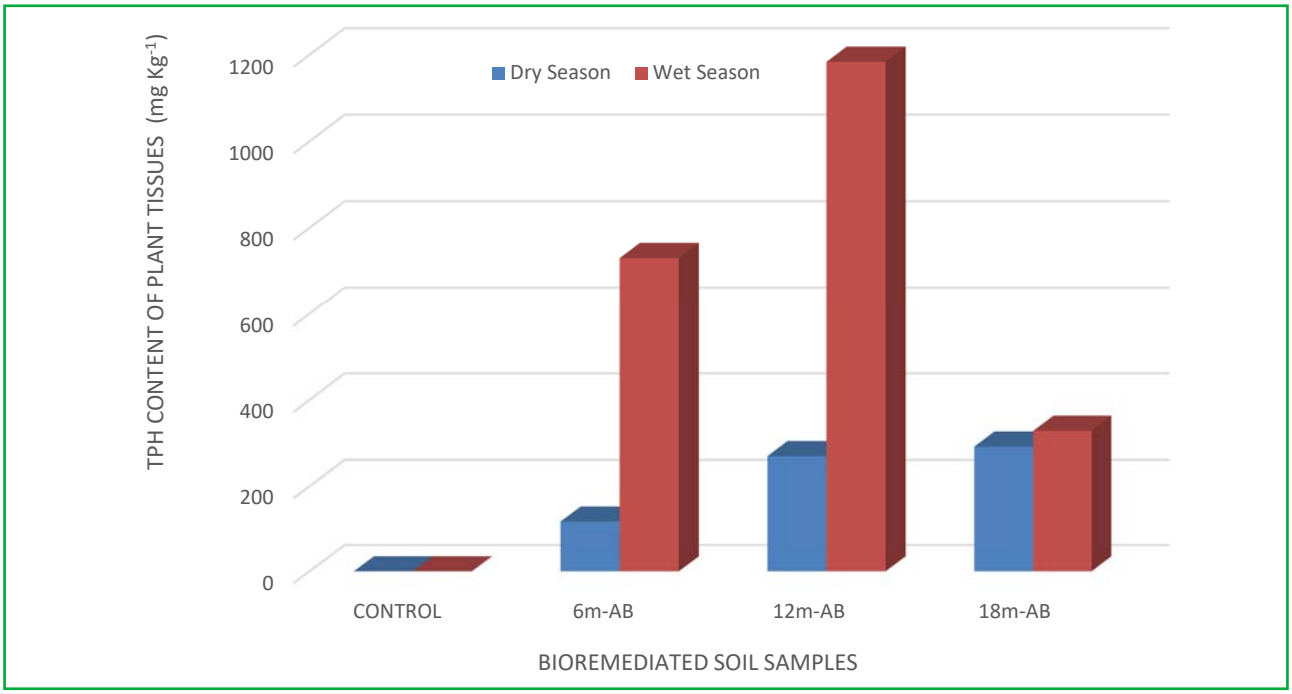

Figure 3 Bioaccumulated TPH content of *Telfairia occidentalis* after growth on various bioremediated crude oil-contaminated soils obtained from Ogoni

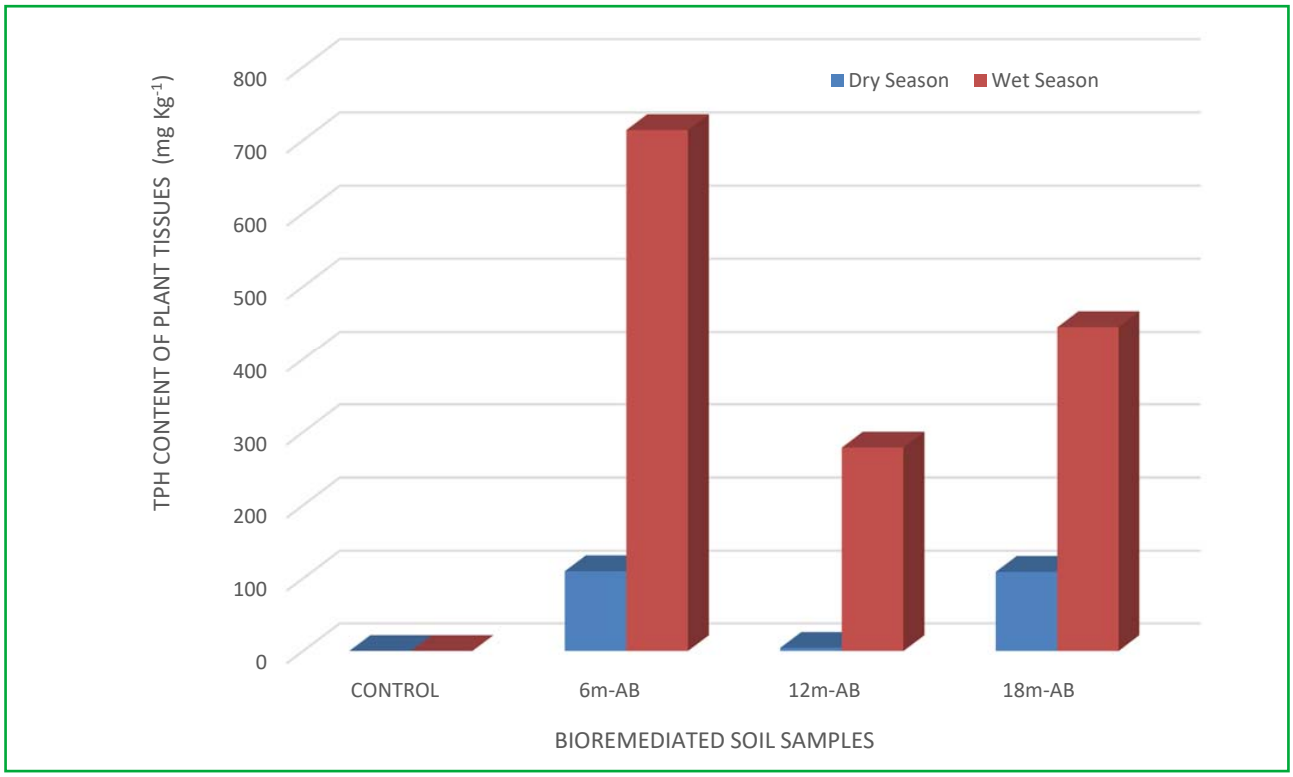

Figure 4 Bioaccumulated TPH content of *Zea mays* after growth on various bioremediated crude oil-contaminated soils obtained from Ogoni

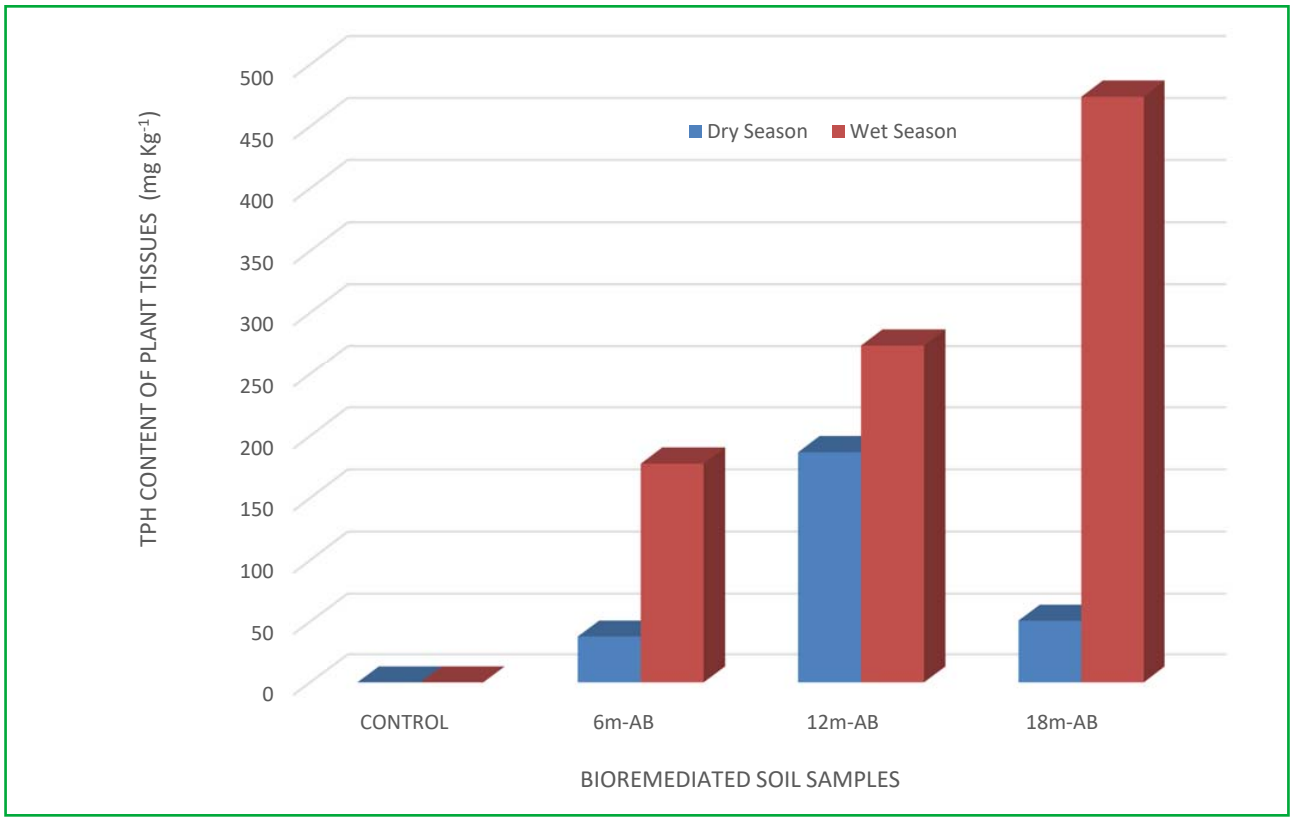

Figure 5 Bioaccumulated TPH content of *Abelmoschus esculentus* after growth on various bioremediated crude oil-contaminated soils obtained from Ogoni

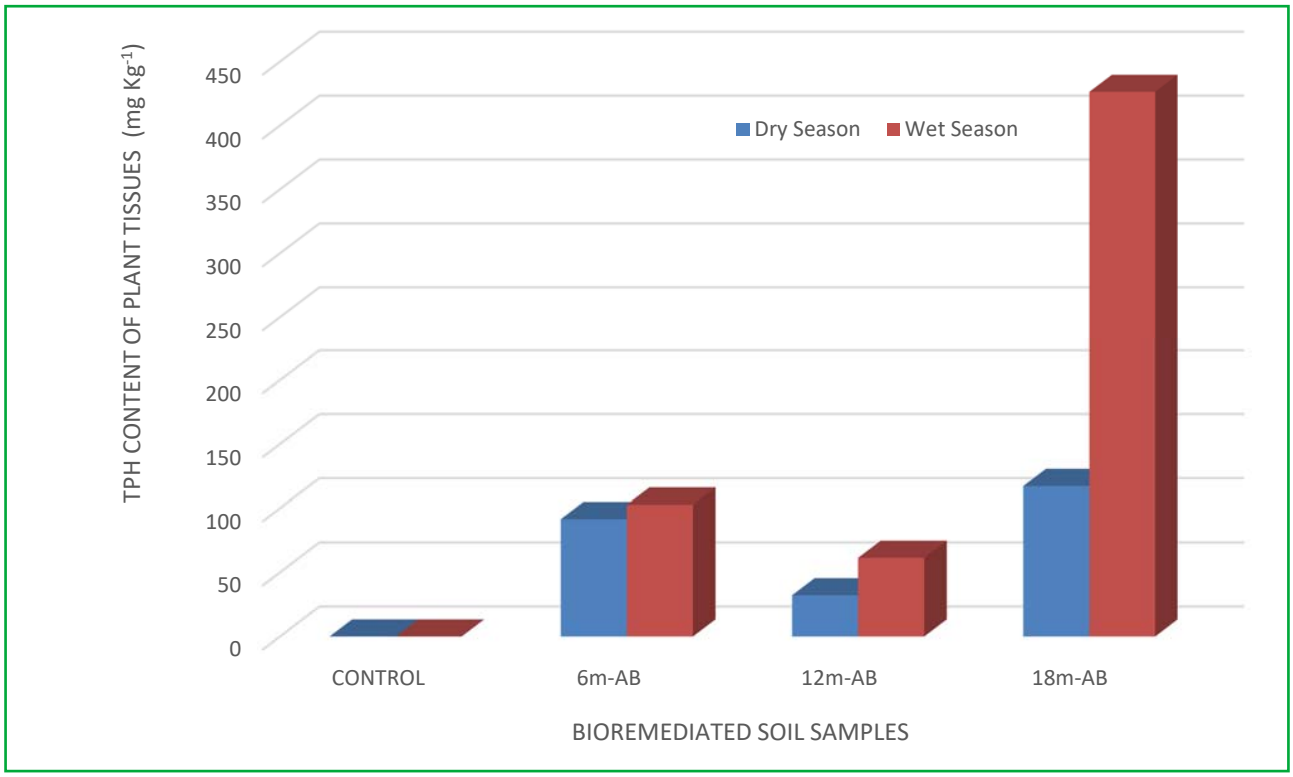

Figure 6 Bioaccumulated TPH content of *Cucumis sativus* growth on various bioremediated crude oil-contaminated soils obtained from Ogoni

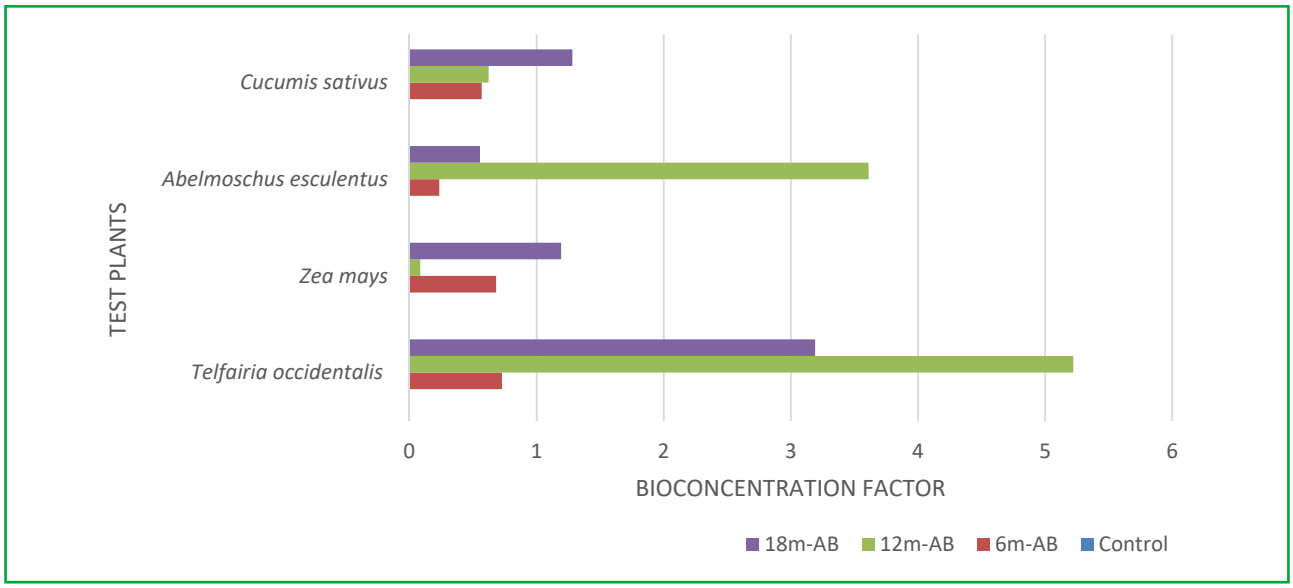

Figure 7 Bioconcentration factor of total petroleum hydrocarbons in tissues of different plants grown on various bioremediated crude oil-contaminated soil samples during the dry season study

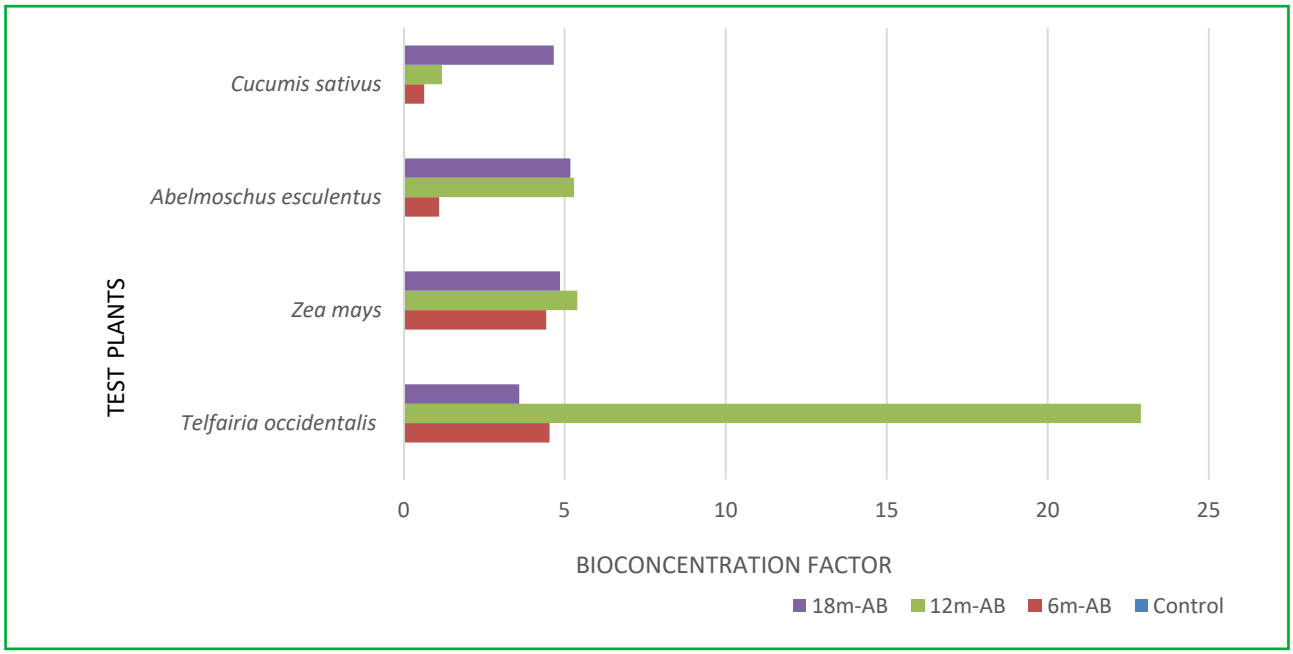

Figure 8 Bioconcentration factor of total petroleum hydrocarbons in tissues of different plants grown on various bioremediated crude oil-contaminated soil samples during the wet season study

and bioconcentration potentials of the test plants. Data obtained during the wet and dry seasons for each plant grown on bioremediated soils showed significant differences (p <0.05) in bioconcentration factor of TPH in tissues. A previous report had stated that TPH uptake and accumulation by plants depend mainly on the type and load of the contaminant in the soil (Lotfinasabasl et al., 2013). Although, the TPH levels in test plants were generally lower than the permissible phytotoxic levels (1,000–12,000 mg kg^{-1}) for plants (Lotfinasabasl et al., 2013), the concern is the resulting growth impairment and the bioconcentration of these contaminants in plants' tissues with time and their eventual transfer to consumers. Adesuyi et al. (2015) had stated that bioconcentration factor values less than one are indicative of plants' limited ability to accumulate, translocate and phytoextract pollutants from soil. A previous study had demonstrated that at concentrations as low as 1,000–1,200 mg kg^{-1}, lighter oils have exhibited phytotoxic effects (Salanitro et al., 1997). The detection of TPH in plants' tissues to this tune is also indicative of the mechanism of uptake by the test plants which is likely more of phyto-stabilization, rather than rhizo-degradation. Phyto-stabilization facilitates the stability of contaminants by immobilizing these chemicals in the soil via absorption, adsorption onto and accumulation into the roots, or via precipitate formation within the rhizosphere. In contrast, when contaminants are broken down in the soil through the bioactive compounds produced and exuded from roots of plants or by soil microflora, then rhizo-degradation is said to have occurred (Lotfinasabasl et al., 2013).

In both dry and wet seasons, the highest bioconcentration factors of 5.22 and 22.88 respectively were obtained for *Telfairia occidentalis* grown on 12m-AB soil. The high magnitude of TPH concentration in the tissues of *Telfairia occidentalis* suggests its high TPH uptake capability and this is worrisome considering its status as a popular vegetable used in preparation of diverse dishes by the locals. The other test plants also exhibited moderate bioconcentration potentials when grown on the bioremediated soils. Such crops grown on bioremediated soil can bioconcentrate harmful contaminants from the soil and transfer same to unsuspecting consumers. In addition, these hydrocarbons may be transferred to livestock, other domesticated animals and wildlife trespassing and grazing on the fauna thriving on such bioremediated soils. The attendant risk involved is the bioconcentration and biomagnification of these contaminants in the animal tissues and their eventual transfer to humans who are non-vegetarians. Apart from foods being likely important sources of exposure to petroleum hydrocarbons in such bioremediated sites, farmers, during their day-to-day work, can also suffer direct exposure from crude oil-contaminated soils (UNEP, 2011). Hence, residual contaminant in the environment pose a significant public health risk to the locals and hence, should not be left alone after being termed recalcitrant. Thus, there is need for risk-based assessment of bioremediated sites before their utilization by locals for safe crop production. This will ensure food produce safety, food security and sustainable development as enshrined in the UN sustainable development goals.

During dry and wet seasons, there was no trace of polyaromatic hydrocarbons (PAHs) in all the four soil samples and plant tissues and loads were obviously lower than the Nigerian regulatory limit of 40 mg kg^{-1} prescribed by DPR (2002). Although, PAHs are low in concentration in fresh Bonny Light crude oil (Little et al., 2018), the non-detection of PAH in the soil was a surprise, Moreover, a previous report has presented PAHs geochemical signature in Bodo between the years 2010 and 2015, and the mean PAH_{16} concentration increased by three folds from 1.30 to 5.65 mg kg^{-1} (Little et al., 2018). Although, crude oil spills arising from several pipeline tappings are chiefly responsible for PAH input in soil, the latter trend was ascribed to the increase in relative concentration during weathering processes, and secondary sources of PAHs such as inputs from combustion of crude oil in illegal refineries which recorded a proliferation after 2009 (Little et al., 2018), pyrogenic emissions from towns, industries in Port Harcourt and Eleme (such as oil refining and petrochemicals and cement, paint and fertilizer) and traffic in the wider Ogoni areas (UNEP, 2011) since oil production, well-testing and flaring activities had been shut down in the area during the period in review. Hence, the presence of PAH in bioremediated soil fallowed for a while from that region was expected. However, the non-detection of PAH in test soils could be due to the highly volatile nature of PAH which may have facilitated their escape into the atmosphere with time. It had been reported that an aged degraded oil sample is almost exhausted of branched and shorter straight chain n-alkanes and the concentration of PAHs is also much reduced (Yang et al., 2012). Moreover, degradation by microbes during reclamation treatment as well as the twin phenomena of dissolution and sorption to soil particles may have depressed their concentration in test soils. Nonetheless, these factors depend on the time the soils were impacted with crude oil, the prevailing conditions and the properties of the PAH hitherto present in the soils. The maximum recommended concentration of the 16 most important PAHs in soil for sensitive human activities (gardening and cultivation of crops for human consumption) in Sweden is 3 mg Kg^{-1} and 20 mg Kg^{-1} for other human activities such as housing (SNV, 2008). Thus, the PAH load in test soils in this study could be adjudged safe for human activities.

The array of hydrocarbon derivatives and other metabolic intermediates in bioremediated soils, uncontaminated soil and tissues of plants grown on all test soils was determined using GC-MS and results obtained are as summarized in Tables 1–5. A higher number of hydrocarbon derivatives/metabolic intermediates were identified in bioremediated soils than in the uncontaminated soil. Most of the derivatives/intermediates found in bioremediated soils were not detected in tissues of plants grown on these soils and this may be attributed to the capacity of these plants to metabolize these derivatives/intermediates to varying degrees without readily retaining such compounds in the tissues. Moreover, these metabolites may have been taken up by plants through soil-plant transfer and converted into other detected toxic compounds in contrast to the plants grown on uncontaminated soil. In addition, it had been stated that the physicochemical characteristics of the hydrocarbons (e.g., molecular weight, hydrophobicity, associated charge, etc.) and the soil characteristics (e.g., texture, soil organic matter, pH, etc.) largely dictate the bioavailability and bioaccessibility of TPH to plants in soil (Chung and Alexander, 2002). Thus, the bioavailability of a particular contaminant will differ from that of another contaminant for a given plant (Reid et al., 2000).

The tissues of the plants grown on bioremediated soils had more of cycloalkanes than straight chain alkanes. Apart from normal and branched alkanes, the next best represented compounds in crude oil are the cycloalkanes, a group of hydrocarbons characterized by their possession of at least one carbon ring. Their occurrence in the tissues of these plants may be attributed to their recalcitrance to biodegradation and their eventual uptake by plants

Table 1 Hydrocarbon compounds and their derivatives determined by GC-MS in soil samples before the study

Organic compounds	Number of variations			
	control	bioremediated crude oil-contaminated soil samples		
		6m-AB	12m-AB	18m-AB
Alkanes	nil	6	4	2
Aromatics	5	4	6	1
Alkenes	nil	1	1	nil
Alkynes	nil	nil	nil	nil
Alcohols	2	7	2	2
Other organics	18	14	20	13

Table 2 Hydrocarbon compounds and their derivatives determined by GC-MS in tissues of *Telfairia occidentalis* grown on various bioremediated crude oil-contaminated soil

Organic compounds	Number of variations							
	at the end of dry season study				at the end of wet season study			
	control	6m-AB	12m-AB	18m-AB	control	6m-AB	12m-AB	18m-AB
Alkanes	4	nil	nil	2	nil	2	2	2
Aromatics	nil	nil	1	nil	2	1	1	nil
Alkenes	5	nil	nil	nil	nil	nil	nil	nil
Alkynes	nil	nil	nil	2	1	nil	nil	nil
Alcohols	8	1	1	1	4	1	nil	nil
Other organics	51	7	4	1	21	20	27	21

Table 3 Hydrocarbon compounds and their derivatives determined by GC-MS in tissues of *Zea mays* grown on various bioremediated crude oil-contaminated soil

Organic compounds	Number of variations Number of variations							
	at the end of dry season study				at the end of wet season study			
	control	6m-AB	12m-AB	18m-AB	control	6m-AB	12m-AB	18m-AB
Alkanes	nil	2	nil	8	nil	1	nil	1
Aromatics	nil	nil	nil	1	nil	1	nil	nil
Alkenes	nil	nil	nil	nil	nil	nil	nil	1
Alkynes	1	nil	nil	1	2	nil	nil	1
Alcohols	2	1	nil	3	3	1	2	1
Other organics	2	6	11	9	11	23	13	15

Table 4 Hydrocarbon compounds and their derivatives determined by GC-MS in tissues of *Abelmoschus esculentus* grown on various bioremediated crude oil-contaminated soil

Organic compounds	Number of variations							
	at the end of dry season study				at the end of wet season study			
	control	6m-AB	12m-AB	18m-AB	control	6m-AB	12m-AB	18m-AB
Alkanes	nil	3	nil	1	1	1	3	2
Aromatics	nil	nil	nil	nil	nil	nil	1	nil
Alkenes	1	2	nil	nil	nil	nil	1	nil
Alkynes	nil	nil	nil	nil	1	nil	nil	nil
Alcohols	2	4	nil	nil	nil	nil	2	1
Other organics	20	20	2	3	28	26	22	21

Table 5 Hydrocarbon compounds and their derivatives determined by GC-MS in tissues of *Cucumis sativus* grown on various bioremediated crude oil-contaminated soil

Organic compounds	Number of variations							
	at the end of dry season study				at the end of wet season study			
	control	6m-AB	12m-AB	18m-AB	control	6m-AB	12m-AB	18m-AB
Alkanes	nil	nil	nil	nil	1	nil	2	2
Aromatics	nil	nil	nil	1	nil	nil	nil	2
Alkenes	nil	nil	nil	1	1	nil	nil	1
Alkynes	nil	nil	1	nil	nil	nil	nil	nil
Alcohols	nil	2	2	2	3	nil	2	nil
Other organics	23	1	3	17	24	14	6	9

where metabolic alteration may also have been resisted. A previous report had stated that highly condensed cycloalkane compounds are resistant to biodegradation due to their physical state and structure. This is mainly because these monocyclic compounds exert a strong solvent effect on membranes consisting of lipids and are toxic to a myriad of hydrocarbon degrading microbes (Bartha, 1986). Some of these hydrocarbon derivatives and metabolic intermediates of concern in bioremediated soils, uncontaminated soil and tissues of plants grown on all test soils are as presented in Tables 6–8. The presence of most fatty acids, aldehydes and alcohols (not detected in control plants) in these tissues may be attributed to the primary attack on intact hydrocarbons (alkanes) which results in generation of alcohols, aldehydes and finally fatty acids in the presence of oxygen (Bartha, 1986).

Many of the intermediate metabolites identified in bioremediated soil samples and plant tissues are efflorescent chemicals that have the capacity to pose serious threat to the health of humans, wild life diversity, terrestrial and aquatic ecosystems (Eggen et al., 2010). Most of these organic compounds – nitrogen containing compounds, phthalate esters, halogenated aliphatic and aromatic compounds are known mutagens and carcinogens (Alimba et al., 2016). They are harmful substances of priority on the list of hazardous substances and are toxic to cells even at minute concentrations (ATSDR, 1997). These intermediate metabolites have also been associated with causation of DNA damage and cytotoxicity in the model cells. For example, identified phthalate esters like diisobutylphthalate and dibutylphthalate in bioremediated soil samples had

Table 6 Some baseline metabolic intermediates in various bioremediated crude oil-contaminated soil and uncontaminated soil obtained from Ogoni

Uncontaminated Soil	6m-AB	12m-AB	18m-AB
5-methyl-4'-hydroxy-2-benzylidene-coumaran-3-one	1,3-bis-t-butylperoxy-phthalan	(sr)- or (rs)-4-methyl-2,3-pentanediol	1-ethenyl-3-(1-hexenyl)-4-trimethylsilylcyclopentane
1-(2-methoxyethoxy)-2-methyl-2-propanol, methyl ether	2,2-dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	1 : 2-phenylisopropanol, tms derivative	2-ethoxy-3-chlorobutane
1,3-bis-t-butylperoxy-phthalan	2,3-dihydro-2-methyl-4-(4-methylphenyl)-1h-1,5-benzodiazepine	2-ethoxy-3-chlorobutane	2-ethyl-3-ketovalerate, 2tms derivative
1,3-oxathiolane-4-carboxylic acid, 2-imino-5-phenyl-, ethyl ester	2,6-dodecadienoic acid, 10-(bromoacetoxy)-11-methoxy-3,7,11-trimethyl-, methyl ester	3,6-bis(trimethylsilyl)-1,4-cyclohexadiene	2-vinylethyl acetate
1h-pyrazole-3-carboxylic acid, 1-[(3-chlorophenyl) methyl]-, methyl ester	2-[4-chloro-trans-styryl]-4-chloropyrimidine	5-(2-methoxypropan-2-yl)-2-methyl-2-vinyltetrahydrofuran	3,4-dimethyl-5-hexen-3-ol
2,3-dichlorothiophene-5-sulfonyl chloride	2-mercaptoethanol, tms derivative	5-methyl-4'-hydroxy-2-benzylidene-coumaran-3-one	5-methyl-4'-hydroxy-2-benzylidene-coumaran-3-one
2-vinylethyl acetate	3,4-dimethyl-5-hexen-3-ol	6,11-dihydro-8-methoxy-1-benzopyrano [4,3-b]indole	9,9-dichloro-9-silafluorene
3-(1,2-dibromoethyl)-1,1,2,2-tetrafluorocyclobutane	3,6-bis(trimethylsilyl)-1,4-cyclohexadiene	9,9-dichloro-9-silafluorene	methyl p-coumarate, tms derivative
3-bromo-2-quinolinecarboxamide	5-methyl-4'-hydroxy-2-benzylidene-coumaran-3-one	butanoic acid, 2-hydroxy-2-methyl-, methyl ester	methylarsine dibromide
3-methoxy-3-methylbutanol	6,11-dihydro-8-methoxy-1-benzopyrano [4,3-b]indole	methyl p-coumarate, tms derivative	trifluoromethyltrimethylsilane
methyl p-coumarate, tms derivative	methyl 10-(chloroacetoxy)-11-methoxy-3,7,11-trimethyl-2,6-dodecadienoate	spiro[(5-bromoacenaphthen-1-one)-2,2'-(5',5'-dimethyl-1',3'-dioxane)]	
methylarsine dibromide	spiro[(5-bromoacenaphthen-1-one)-2,2'-(5',5'-dimethyl-1',3'-dioxane)]	trifluoromethyltrimethylsilane	
trimethyl (3,3-difluoro-2-propenyl)silane	trimethyl (3,3-difluoro-2-propenyl)silane	trimethyl (3,3-difluoro-2-propenyl)silane	

Table 7 Some metabolic intermediates in tissues of various plants grown on bioremediated crude oil-contaminated soils from Ogoni during the dry season

Telfairia occidentalis	*Zea mays*	*Abelmoschus esculentus*	*Cucumis sativus*
1,2-propanediamine	2-tridecen-1-ol, (e)-	1,1,1,3,5,5,7,7,7-nonamethyl-3-(trimethylsiloxy)tetrasiloxane	cyclopropyl carbinol
acetic anhydride	naphthalene, decahydro-2-methyl	2-aminononadecane	1,2-propanediamine
ethylene oxide	hexadecane	2-bromononane	acetamide
7-nitroimino 2,4,6,8-tetraazabicyclo[3.3.0] octan-3-one,	pentadecane	3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	cyclopropyl carbinol
3,7-diacetamido-7h-s-triazolo[5,1-c]-s-triazole	trans-2-dodecen-1-ol	3-methyl-3,5--(cyanoethyl) tetrahydro-4-thiopyranone	ethyne, fluoro
cyclohexanecarboxylic acid, octyl ester	(2-aziridinylethyl)amine	acetic acid, 2-(1-methyl-2-oxohydrazino)-, n′-[(e)-(2-hydroxyphenyl)methylidene] hydrazide, n-oxide	1-cyclohexylnonene
n-propyl heptyl ether	1,4-dioxane-2,6-dione	catechol, 2tbdms derivative	2,2,3,3,4,4-hexamethyltetrahydrofuran
bicyclo[4.2.1]nona-2,4-dien-9-endo-ol, 9-exo-ethenyl	1-octanamine, n-methyl-	cycloheptasiloxane, tetradecamethyl	2,4-azetidinedione, 3,3-diethyl-1-methyl
cyclododecanol	n-hexylmethylamine		oxacyclododecan-2-one
nonadecane	1-methyldecahydronaphthalene	decane, 1-iodo	oxirane, tetradecyl
panaxjapyne a	1-octadecyne	dispiro[2.2.2.2]deca-4,9-diene	pyrazole, 5-cyclohexylamino-1,3-dimethyl-4-nitro
	2,6-dimethyldecane	endo-3-methylenetricyclo[3.2.1.0(2,4)] oct-6-ene	2,2,3,3,4,4-hexamethyltetrahydrofuran
	decane	heptane, 4-azido	1-cyclohexylnonene
	dodecane	1,3-dioxane-4,6-dione, 2,2-dimethyl	2,4-azetidinedione, 3,3-diethyl-1-methyl

Table 8 Some metabolic intermediates in tissues of various plants grown on bioremediated crude oil-contaminated soils obtained from Ogoni during the wet season

Telfairia occidentalis	*Zea mays*	*Abelmoschus esculentus*	*Cucumis sativus*
lanosta-7,9(11)-dien-18-oic acid, 22,25-epoxy-3,17,20-tri-hydroxy-, γ-lactone, (3β)	(14β)12,13-epoxyolean-3-ol, acetate	(5β) pregnane-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	n-(methoxy-methyl)piperidine
1,2-benzisothiazol-3-amine, tbdms derivative	(6β,11β,16β)-6,11,21-tri-hydroxy-2'-methyl-5'h--pregna-1,4-dieno[17,16-d] oxazolo-3,20-dione	1h-cyclopropa[3,4]benz[1,2--e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro--3-(hydroxymethyl)-1,1,6	tuaminoheptane
3-isopropoxy-1,1,1,5,5,5-he-xamethyl-3-(trimethylsiloxy) trisiloxane	1-{2-(4-chlorophenyl)-2-[(tri-methylsilyl)oxy]ethyl}-4-(4-flu-orobenzyl)piperidine	3-isopropoxy-1,1,1,7,7,7-hexa-methyl-3,5,5-tris(trimethylsilo-xy)tetrasiloxane	9-octadecenoic acid (z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester
6,6,8,8-tetramethyl-2,5,7,9,12--pentaoxa-6,8-disilatridecane	17,20-isocholest-5-en--3β,16,26-triol	5-(4,5-dihydro-3h-pyrrol-2-yl-methylene)-4,4-dimethylpyrro-lidine-2-thione	ethyl iso-allocholate
disiloxane, 1,3-diethoxy--1,1,3,3-tetramethyl	18,19-secoyohimban-19-oic acid, 16,17,20,21-tetrade-hydro-16-(hydroxymethyl)-, methyl ester, (15β,16e)-	6-hydroxy-4,4,5,6-tetrame-thyltetrahydro-1,3-thiazin--2-thione	2-propanamine, 1-methoxy
octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13, 13,15,15-hexadecamethyl	1-methyl-1-(7-tridecyl) oxy-1-silacyclopentane	arsenous acid, tris(trimethylsi-lyl) ester	butane, 2-methoxy-2,3,3-trimethyl
silane, cyclohexyldimethoxymethyl	2,4-di-tert-butyl-6-(tert-butyla-mino)phenol	cyclohexasiloxane, dodecamethyl-	cyclopentane, (2-methylpropyl)
silane, dimethoxydimethyl	3-[4-bromobenzoyl] aminobenzamide	oxirane, [(1-methylethoxy) methyl]-	cyclopentane, 1-methyl-3-(1-methylethyl)
tris(tert-butyldimethylsilyloxy) arsane	4'-(4-morpholino-1,8-naphtha-limido)morpholinophenone	tris(tert-butyldimethylsilyloxy) arsane	1,2-ethanediamine, n-methyl
1-di(tert-butyl) silyloxy-2-phenylethane	batrachotoxinin a, 7,8-dihyd-ro-o3-methyl-, (8β,20.xi.)-	2-(16-acetoxy-11-hydroxy--4,8,10,14-tetramethyl-3-oxo-hexadecahydrocyclopenta[a] phenanthren-17-ylidene	1,2,4,5-tetrazine, 3,6-dimethyl
1-nitro-β-d-arabinofuranose, tetraacetate	2,4-di-tert-butyl-6-(tert-butyla-mino)phenol	2,4-cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)--4-hydroxy	9,9-dichloro-9-silafluorene
2-pentene, 3-(chloroethylbory-l)-2-(chlorodimethylsilyl)-, (e)-	3-hexene, 1-(1-ethoxyethoxy)-, (z)-	6h-benzofuro[3,2-c][1]benzo-pyran, 3,9-dimethoxy	hydrogen azide
2-trifluoromethyl-7-phenothi-azone	4-chlorobuten-3-yne	3-isopropoxy-1,1,1,7,7,7-hexa-methyl-3,5,5-tris(trimethylsilo-xy)tetrasiloxane	isobutane
3(2h)-benzofuranone, 6-me-thoxy-2-[(4-methoxyphenyl) methylene]-, (e)-	benzeneethanamine, 4-benzy-loxy-3-fluoro-β-hydroxy-5-me-thoxy-n-methyl	n,n-dimethyl-4-nitroso-3-(tri-methylsilyl)aniline	aziridine, 1-methyl
4-(5-chloro-2-methoxyphenyl)--5-cyclopropyl-1,2,4-triazole--3-thiol	bromacil	pregn-5-ene-3,11-dione, 17,20:20,21-bis[methylenebis-(oxy)]-, cyclic 3-(1,2-ethanediyl acetal)	methanamine, n-butylidene
4,4'-isopropylidene-bis(2--chlorophenol)		benzaldehyde, 2-nitro-, diaminomethylidenhydrazone	propiolactone
rhodopin		bicyclo[2.2.1]heptane-2,3--diol, 1,7,7-trimethyl-, (2-endo,3-exo)-	
satratoxin h		n-desmethyltapentadol	

induced DNA single-strand breaks in nasal mucosa and pharyngeal epithelia cells as reported by Kleinsasser et al. (2000). Likewise, DNA double-strand breaks were induced by Bisphenol A in mutant chicken DT40 cell line deficient in DNA repair pathways (Lee et al., 2013). Trifluoromethyltrimethylsilane, another metabolite detected in 18m-AB soil and plants on same soil, is known to cause central nervous system depression, eye and skin irritation as well as respiratory irritation in humans when exposed to this chemical (Federal Register, 2012). Furthermore, amongst these metabolites found in the bioremediated soil samples are stable lipophilic compounds that may have the ability to bioaccumulate in plant tissues. This will eventually lead to their biomagnification via food webs through accumulation in fat-rich tissues of higher trophic animals with humans inclusive. Consumption of these food crops can lead to cancers as well as other ailments as reported by Khan et al. (2008). Accumulation of organic compounds in edible vegetables grown on bioremediated soils had been reported in a previous study by Shagal et al. (2012). The presence of these contaminants in the soil also led to contamination of underground and surface potable water supply around the area where the oil spill occurred according to Someya et al. (2010) and Melnyk et al. (2014). This is an indication that water sources around contaminated sites, bioremediated soils and edible crops grown on such soils may be possible human exposure routes to these mixtures of chemicals. Studies have shown that people living around and working in landfill facilities had higher concentrations of deleterious organic pollutants and heavy metals in their breast milk and blood than control population (Devanathan et al., 2012) and so similar scenario could play out in bioremediated soils exposed to humans in one way or the other. Moreover, some of the compounds identified in bioremediated soil samples are known endocrine disrupting chemicals which are capable of reproduction and developmental processes impairment resulting in increased cases of carcinogenesis as reported by Eggen et al. (2010).

Based on the array of hydrocarbon derivatives/intermediates detected in soil and their obvious toxic, mutagenic and carcinogenic potentials, it is opined that bioremediated crude oil-contaminated sites in Ogoni studied are clearly not suitable for crop cultivation, human activities and the ecosystem supporting them. More worrisome is the fact that, unlike TPH and PAH, there are no environmental quality standards for these hydrocarbon derivatives/intermediates for purposes of regulation and enforcement after conclusion of remediation projects. To this end, to ensure eco-safety, there is need to derive an EQS scientific-wise to be used for comparison with analytical results in order to put this subjective assessment in a comprehensive context. However, it is pertinent to state that factors such as chemistry, epidemiology, toxicology, metabolism, chemical interaction between compounds, and other factors that in combination affect the probability of adverse effect are required for derivation of an EQS for chemical substances and hence, is a sophisticated process (Little et al., 2018). A clear and concise definition of the ecological goals is required for ecological restoration of crude oil-contaminated soils and this needs detailed comprehension of the array of hydrocarbon derivatives and metabolic intermediates arising from microbial activities, their toxicity mechanism and tolerance of inter-dependent species, communities and habitats to the perturbation.

4 Conclusions

Data from this study show that the TPH content of the bioremediated crude oil-contaminated soil samples varied widely from that of pristine soil as TPH content was below detectable limits in the uncontaminated pristine soil. In bioremediated soils, TPH content was above the target value of 50 mg kg^{-1} recommended by EGASPIN (DPR, 2002). However, PAHs were below detectable limits in both uncontaminated and bioremediated soils. A wide array of petroleum-derived metabolic intermediates was identified in bioremediated soils when compared to the uncontaminated pristine soil. Some of the identified metabolites and residual contaminants are known carcinogens and eco-toxicants and may be deleterious to plant growth, animals and humans thus, suggesting the unhealthy status of the studied bioremediated soils for safe crop production. Thus, this study highlights the need for suitable assessment of contaminant residues and metabolic intermediates in remediated soils before commencement of farming activities in order to ensure the production of safe crops from such reclaimed polluted sites.

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