Physicochemical Profiles of Various Bioremediated Petroleum Contaminated Sites in Ogoniland, Nigeria.

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Abstract

This study was carried out to evaluate the physicochemical statuses of bioremediated sites in Ogoniland (K-Dere, Bodo, and Biara), Rivers State, Nigeria after a certified bioremediation protocol. Three bioremediated soils of different fallow ages (6 months, 12 months, and 18 months after bioremediation-AB) and an uncontaminated soil (Bera) were collected and analyzed for various parameters- pH, electrical conductivity (EC), total organic matter (TOM), particle size distribution, cation exchange capacity (CEC), nitrogen and phosphorus, heavy metals (lead, cadmium, nickel and copper), total petroleum hydrocarbons (TPH), poly aromatic hydrocarbons (PAH), and intermediary metabolites. Results obtained indicate that the particle size distribution of the four soil samples were similar in terms of their content of sand, silt and clay. The pH of 12m-AB and 18m-AB bioremediated soil samples were 6.34 and 6.50 respectively and were slightly lower than pH of uncontaminated soil. The EC as well as the CEC of the bioremediated soil samples were considerably lower when compared to the uncontaminated soil sample. The range of values for TOM was between 0.095 – 1.232 % with 6m-AB soil having the least value; whereas 0m-AB and 12m-AB had the highest value of 1.232 %. Sample 18m-AB had the highest concentration of nitrogen whereas, 12m-AB sample had the least concentration. The phosphorus content in each bioremediated soil was significantly lower than in uncontaminated soil. The residual TPH content of each bioremediated soil sample was above the recommended EGASPIN target TPH value of 50 mg/kg but below the intervention level of 5,000 mg/kg. The TPH contents in bioremediated soil samples were 161.25 mg/kg (6m-AB), 51.72 mg/kg (12m-AB) and 91.50 mg/kg (18m-AB). TPH was not detected in the uncontaminated soil sample. All four samples had no trace of PAH. Heavy metals were below detectable limits in all soil samples. Screening of the soil samples using gas chromatography-mass spectrometer revealed a number of metabolic intermediates in bioremediated soil samples when compared to the uncontaminated pristine soil (control). Some of the identified metabolites are known carcinogens and are deleterious to plant growth thus suggesting the unhealthy status of the bioremediated soils for agricultural productivity.

Key words: bioremediated sites, TPH, metabolites, heavy metals, soil

Introduction

Presently, the contamination of the natural environment by petroleum hydrocarbons is of enormous concern and the ever-increasing demand for petroleum products by emerging economies has worsened the problem. In other to meet this demand, there has been increased exploration of petroleum reservoirs leading to a surge in oil spills arising from activities like extraction, processing, transportation and storage of petroleum and petroleum products in the oil industry (Olguín et al., 2007). Levy et al. (2010) and 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 petroleum 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 contaminants from soil with possible effects on living organisms (Ortínez et al., 2003). Exposure to petroleum can lead to noticeable alteration in physical and chemical features of the soil, limit microbial growth and retard plant development (Vázquez-Luna, 2012).

Certain biological and environmental parameters determine the success of bioremediation strategies aimed at restoring these hydrocarbon polluted environments to their natural state prior to contamination. Sathishkumar et al. (2008) listed some of these parameters to include nutrients, temperature, pH and microorganisms present while Joo et al. (2008) mentioned bioaccessibility/bioavailability of the contaminant, chain length and class of the polluting hydrocarbon. It is easier to put these parameters in check to ensure an effective bioremediation process than to combat the deleterious effect of hydrocarbons and degradation intermediates that arise during or after the process.

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 petroleum contamination does not always translate to decrease in soil toxicity. Soil toxicity could be worsened by the emergence of intermediary 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 being investigated. 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 (Jarup, 2003; Khan et al., 2008; Abbas et al., 2011).

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 intermediary 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.

The use of conventional chemical analysis gives no information about these intermediates as it only gives information about the concentration of residual contaminants. Hence, in this study three (3) petroleum contaminated soil samples that had been certified bioremediated by the relevant regulatory agencies were subjected to gas chromatography-mass spectrometry analysis to determine the array of inherent metabolic intermediates in the samples. Data from this investigation revealed the chemical load of these remediated soil samples.

Materials and Methods

Collection of Soil Samples

Soil samples were collected from bioremediated petroleum hydrocarbon contaminated sites in Ogoniland, Rivers State, Nigeria in October, 2018 for this study. The sampling location is presented in Fig. 1. The bioremediated sites were selected based on their ages (6 months, 12 months, and 18 months after intervention) following remediation certification. Another soil sample was collected from an unpolluted area with undisturbed soil which served as the control. The bioremediated and uncontaminated (control) soil samples obtained were sandy loam in texture. Soil type was young sedimentary soil (histosols) derived from recent alluviation (Onyeike et al., 2002). Surface soil (0–30 cm depth) was taken from each selected bioremediated site and control site using a Dutch soil auger. Forty (40) soil samples were collected at all sampling sites to make four composite samples. Composite sampling was carried out at each of the sites to have a good representation of each sampling site. A fixed grid reference was drawn up for each bioremediated and control site visited and the sampling design involved the utilization of random numbers. At individual sites, the sample size (number of soil samples collected) for each composite sample was ten. Rocks and other particles in the soil samples were removed before mixing to make the composite samples. The composited samples were stored in clean, sterile polythene bags and thereafter, sent to the laboratory for physicochemical analyses in an ice box maintained at a temperature of about 4^{o}C.

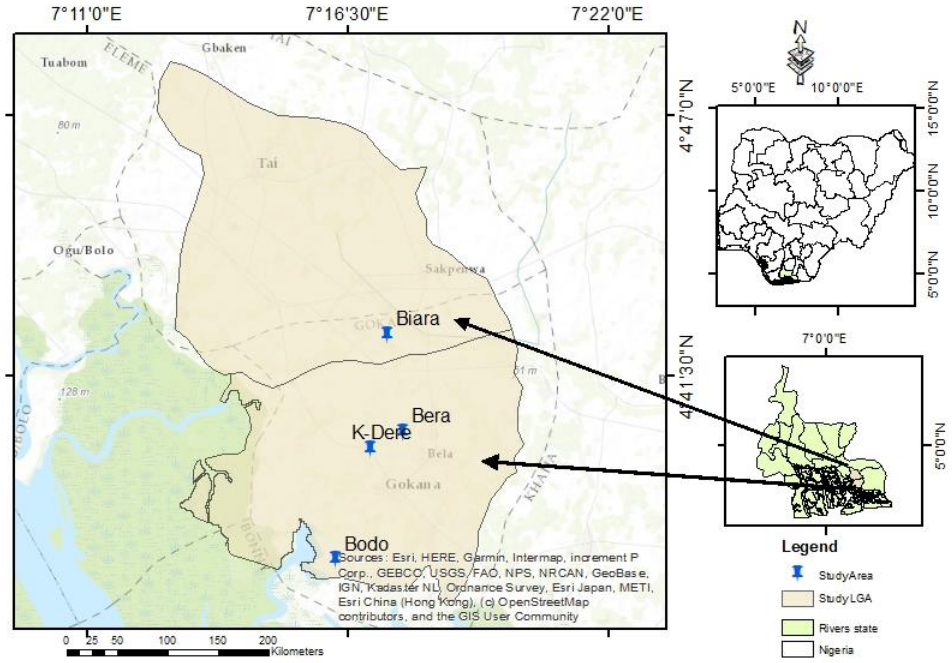

Fig. 1. Map of locations in Ogoniland sampled in this study

Physicochemical Analyses of Soil Sample

Soil pH was determined using a pH meter (Mettler Delta) while the electrometric method was used to determine the electrical conductivity of the soil samples. ASTM D2579 method and ASTM 7503 method were employed to determine the total organic matter and Cation Exchange Capacity respectively. Particle Size Distribution was determined using the ASTM 6913 method while nitrogen content of samples was determined colorimetrically by UV 1800PC spectrophotometer in accordance with EPA 352.1 and APHA 4500-NO_3^- B. Phosphate was also determined colorimetrically by UV 1800PC spectrophotometer in accordance with APHA 4500-P-D and Stewart (1989). Total petroleum hydrocarbon (TPH) concentration in the samples were determined with Varian CP 3800 gas chromatography (GC) in accordance with ASTM D5765, EPA 1625 and USEPA 8270B. Polycyclic aromatic hydrocarbon (PAH) content and metabolic intermediates in soil samples were identified using Agilent 6890 GC-MS in accordance to ASTM D7363. For the heavy metals, methods employed were ASTM D8064 (nickel and copper); ASTM D3559 (lead) and ASTM D3557 (chromium) using a flame atomic absorption spectrophotometer.

Data obtained were subjected to statistical analysis using the one-factor Analysis of Variance (ANOVA) to determine if there are significant differences between data obtained from different sites.

Results and Discussion

The physicochemical characteristics of three bioremediated petroleum contaminated soils and an uncontaminated pristine soil obtained from Ogoniland, Rivers State are as presented in Table 1. The three soil samples (6m-AB, 12m-AB and 18m-AB) collected from a crude oil spill site had been certified bioremediated 6 months, 12 months and 18 months prior to sampling respectively by regulatory agencies after an intervention by reclamation outfits. The texture of the bioremediated petroleum contaminated soils and control soil are as presented in Table 1. In all four soils, generally, the sand content was twice higher than the

silt content with clay content being the least. The particle size distribution analysis shows that all soil samples are sandy-loam soil as the proportion of sand ranged from 68.00 – 65.60%; silt between 22.60 – 19.70 %, whereas the clay content in the four soil samples ranged from 14.50 – 10.70 %. The particle size distribution of the four soil samples were similar in terms of their content of sand, silt and clay since they were obtained from the same locality in Ogoniland. Soil texture influences the physical parameters of the soil and plays a very important role in microbial and plant species establishment and development (Chau et al., 2011; Daryanto et al., 2016). The higher percentage of sand and silt in the soil samples suggests adequate porosity and aeration which ensures deleterious contaminants are eliminated through biodegradation and vertical migration (Haghollahi et al., 2016). Colak (2012) had reported low adsorption soil capacity due to low proportion of clay as well as low organic carbon content.

The concentration of nitrogen in soil samples ranged from 73.49 – 35.34 mg/kg with 18m-AB sample having the highest concentration of nitrogen while 12m-AB sample had the least concentration. Inundation of adjoining farmlands during flood events may be responsible for the elevated nitrogen levels in 18m-AB soil sample via horizontal transfer. Sustained use of NPK fertilizer for crop production in farms allows for transfer of these elements to adjoining lands during rainfall events being a consequence of the site's topography. Phosphorus levels in the four soil samples were statistically different ($p<0.05$) and ranged from 0.218 to 0.571 mg/kg. Pristine soil (control) sample had the highest concentration (0.571 mg/kg) of phosphorus while sample 18m-AB had the lowest concentration of phosphorus (0.218 mg/kg). The concentrations of extractable macronutrients, phosphorus, in the bioremediated soil were significantly lower than in uncontaminated soil (Table 1). These relatively lower concentrations of phosphorus could be attributed to its uptake by resident microflora in soil during biodegradation of residual hydrocarbons. Nitrogen and phosphorus are key elements needed for synthesis of proteins, enzymes and nucleic acid during growth of microbes on hydrocarbons. They also influence nutrient-induced community dynamics of native microorganisms and their metabolic interplay (Roy et al., 2018). Oil spill events exert a pressure on the C:N and C:P ratios in soil due to an excessive input of carbon compared to the available micronutrients which facilitates the assimilation of nitrogen and phosphorus by microbes (Xia et al., 2006; Sutton et al., 2013). These two elements are required for eventual utilization of the hydrocarbons as carbon sources and for energy generation.

The pH of 12m-AB and 18m-AB bioremediated soil samples were 6.34 and 6.50 respectively and were slightly lower than pH of uncontaminated pristine soil (7.48) and 6m-AB bioremediated soil (7.40). Bioconversion of residual hydrocarbons to organic acids even after validation of bioremediated sites may have led to the slight acidity of the bioremediated soil samples. Moreover, the TPH and heavy metal contents of crude oil could lead to impairment of gaseous exchange and retention of soil carbon dioxide (Ujowundu et al., 2011). These conditions might have resulted in decreased porosity and conductivity and increased acidity of the bioremediated soils in this study. Soil buffering activities during the bioremediation protocol may be responsible for the neutral pH (7.40) of 6m-AB soil sample. Soil pH has a great influence on microbial activity and ultimately bioremediation success or rate as most microorganisms can thrive only within a certain pH range. For instance, neutral

pH of 7 might be optimal for the degradation of chemicals by microbes however, pH range in the order of 6–8 may also be acceptable for effective microbial attack. The pH of soil samples used in this study fell within this range. Rousk et al. (2010) had observed high microbial activity in neutral soils and lower microbial activity in acidic soils whereas, Itah and Essien (2001) had reported bacteria that thrive in alkaline or acidic soil condition. In a study carried out by Mukut and Arundhuti (2012), soil samples whose pH was adjusted to 4.5 and 7.5 had significant biodegradation of petroleum hydrocarbons unlike soil samples whose pH was adjusted to 3.5. Depending on other soil features, a change in pH of a soil contaminated with petroleum may cause contaminants to precipitate and become highly mobile or make contaminates to adsorb to the soil and inhibit degradation (Ajoku and Oduola, 2013). Previous reports have shown that pristine tropical garden soil generally has a pH of about 7.15 (Choppala et al., 2018). A study by Verstrate et al. (1975) emphasized optimal activity for microbial degradation at a pH of 7.4 and considerable inhibition at pH 4.5 and 8.5. Nevertheless, the pH of the three bioremediated soil samples in this study were within the range of 6 and 8 which suggests that bacterial degradation activities will be sustained provided favorable conditions exist in soil. Favorable soil pH is important in ensuring sustained microbial metabolism in soil in order to facilitate the extinction of the residual hydrocarbons in the soil (Atlas and Bartha, 1992). Moreover, nutrient availability in soil or water may be influenced by pH.

The electrical conductivity of the bioremediated soil samples was considerably lower when compared to the uncontaminated soil sample. Pristine soil had the highest electrical conductivity value of 8.00 µs/cm while 6m-AB and 12m-AB soil had the least value of 2.00 µs/cm. Likewise, the concentrations of sodium, calcium and magnesium were lower than was obtained for the uncontaminated soil sample thus, suggesting that petroleum contamination and eventual biological treatment of the soil altered the soil's physicochemical characteristics. The cation exchange capacity (CEC) comprises of four (4) compounds which are potassium, sodium, calcium, and magnesium. For sodium, pristine soil had the highest value (0.4613 mg/kg) while 18m-AB had the lowest value (0.3056 mg/kg); for calcium, the highest value of 2.864 mg/kg was obtained from pristine soil sample while the least value of 0.041 mg/kg was obtained from 12m-AB sample; for magnesium, pristine soil had the highest value of 0.3831 mg/kg whereas the least value (0.1438 mg/kg) was obtained from 12m-AB. Potassium was not detected in uncontaminated and bioremediated soil samples. The reduction in the concentrations of sodium, calcium and magnesium which are essential nutrients and suitable terminal electron acceptors may have affected the indigenous microbial growth and metabolism (Ujowundu et al., 2011). The lower conductivity values of 2.0 to 4.0 µS/CM in bioremediated soils when compared to 8.0 µS/CM in the uncontaminated soil may be attributed to the reduction in the nutrient content of the bioremediated soils. There is a positive correlation between available nutrient and electrical conductivity. The range of values for total organic matter (TOM) was 0.095 – 1.232 % with 6m-AB soil having the least value; whereas pristine soil and 12m-AB had the highest value of 1.232 %. There were no significant differences ($p=0.05$) between the total organic matter (TOM) content of the uncontaminated soil and the bioremediated soil samples though, the TOM in uncontaminated soil was slightly higher than was obtained in bioremediated soil samples. The annihilation of plant species soon after the oil spill that occurred in bioremediated sites and the enhanced

microbial activities as a result of the bioremediation protocol may have led to the drop in TOM content of these soil samples.

Table 1. *Physicochemical Characteristics of Soils obtained from Ogoniland, Rivers State.*

Parameters		Sample Identity			
		Control	**6m-AB**	**12m-AB**	**18m-AB**
pH		7.48	7.40	6.34	6.50
Electrical Conductivity, EC (µS/CM)		8.00	2.00	2.00	4.00
Total Organic Matter, TOM (%)		1.232	1.095	1.232	0.958
Cation Exchange Capacity, CEC	Potassium, K (mg/kg)	<0.001	<0.001	<0.001	<0.001
	Sodium, Na (mg/kg)	0.4613	0.3468	0.3646	0.3056
	Calcium, Ca (mg/kg)	2.864	0.102	0.041	0.10
	Magnesium, Mg (mg/kg)	0.3831	0.1663	0.1438	0.1948
Particle Size Distribution, PSD (%)	Sand	65.60	68.4	65.80	66.80
	Silt	22.60	20.60	19.70	22.50
	Clay	11.80	11.00	14.50	10.70
Nitrogen (mg/kg)		38.007	46.940	35.340	73.490
Phosphorus (mg/kg)		0.571	0.480	0.283	0.218
Total Petroleum Hydrocarbon, TPH (mg/kg)		BDL	161.25	51.72	91.50
Polyaromatic Hydrocarbon, PAH (mg/kg)		BDL	BDL	BDL	BDL

The TPH contents in bioremediated petroleum contaminated soil samples encountered were 161.25 mg/Kg, 51.72 mg/Kg and 91.50 mg/Kg for 6m-AB, 12m-AB and 18m-AB respectively. These values exceed the EGASPIN recommended TPH target value of 50 mg/kg however, they are below the intervention level of 5,000 mg/kg. The uncontaminated pristine soil sample had no trace of residual TPH. The higher-than-target value residual TPH content obtained for these bioremediated soils suggests that the reclamation process fell short of achieving the set objectives of regulatory agencies. The relatively higher TPH concentration obtained in 6m-AB could be attributed to its age status (6 months after intervention) when compared to the samples obtained from other sites that had fallowed for 12 months and 18 months after intervention. During site fallowing, degradation of residual petroleum hydrocarbons is sustained by surviving microorganisms as long as conditions remain favorable for their growth despite site demobilization. This may account for lower residual TPH values obtained for 12m-AB and 18m-AB. TPH degradation may happen naturally by native microorganisms with degradation rate of 77% after 30 months of contamination (Rhykerd et al., 1999). Biodegradation of target compounds by indigenous microbial communities is frequently considered to be the primary mechanism for attenuation of contaminants (Declercq et al., 2012). Stimulated or not, indigenous microorganisms capable of petroleum hydrocarbon biodegradation could have a crucial impact on remediation, especially if site was exposed prior to contamination (Bento et al., 2005; Sabaté

et al., 2004). However, the composition of the hydrocarbons and the prevailing environmental conditions affect the composition and competence of the inherent microbial population (Bento et al., 2005). Hence, concentrations of the TPH obtained in sites studied despite being certified reclaimed can render conditions in soil unsatisfactory for the growth of plants and microorganisms.

Even more worrisome is the load of intermediate metabolites arising from the degradation of TPH by microbes. Screening of the soil samples using gas chromatography-mass spectrometer revealed a number of metabolic intermediates in bioremediated soil samples when compared to the uncontaminated pristine soil (control). The array of metabolites identified is as presented in Table 2. Some of these metabolites are known carcinogens and are deleterious to plant growth. Their presence in the bioremediated soil is indicative of the unhealthy status of the soils in terms of agricultural productivity. One of the main aims of polluted site reclamation especially in Nigeria is the resumption of farming activities and animal husbandry on such sites (UNEP, 2011; Umukoro, 2012) since most locals in affected areas depend on agriculture for survival. Hence, the presence of these metabolites in the reclaimed petroleum contaminated sites may hinder plant growth and agricultural productivity.

Most of the metabolites detected in bioremediated soil samples are efflorescent chemicals with the capacity to pose a threat to human health, aquatic and terrestrial ecosystems and wild life diversity (Eggen et al., 2010). Many of these organic compounds-halogenated aliphatic and aromatic compounds, nitrogen containing compounds, and phthalate esters, are known carcinogens and mutagens (Alimba et al., 2016). They have been prioritized on the list of hazardous substances and are deleterious to cells even at minute concentrations (Fay and Mumtaz, 1996; ATSDR, 1997). These metabolites have also been implicated in the causation of cytotoxicity and DNA damage in the model cells. For instance, dibutylphthalate and diisobutylphthalate (phthalate esters) detected in soil samples in this study had induced DNA single-strand breaks in nasal mucosa and pharyngeal epithelia cells in previous reports (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). Other metabolites like trifluoromethyltrimethylsilane is known to cause skin and eye irritation, respiratory irritation, and central nervous system depression in humans when exposed to this chemical (Federal Register, 2012). Moreover, some of these metabolites present in the bioremediated soil samples are stable lipophilic compounds and hence may possess bioaccumulation potentials in plants which will result in their biomagnification through food webs via accumulation in fat-rich tissues of higher trophic animals including humans. Consumption of such food materials may result in cancers and other ailments (Jarup, 2003; Khan et al., 2008; Abbas et al., 2011). A previous report had shown that organic compounds in bioremediated soils were readily accumulated in cultivated edible vegetables (Shagal et al., 2012), and also led to contamination of underground and surface potable water supply around the locality of the oil spill sites (Melnyk et al., 2014; Sanchez-Chardi et al., 2007; Someya et al., 2010). This suggests that bioremediated soils, water sources around contaminated sites and edible crops cultivated on such soil may be possible human exposure routes to these chemical mixtures. It has been shown in previous reports that people working in and living around landfill facilities harbored higher concentrations of toxic metals and

organic pollutants in their blood and breast milk than control population (Devanathan et al., 2012; Malarvannan et al., 2009). More so, some of these compounds detected in bioremediated soil samples may be endocrine disrupting chemicals capable of impairing reproduction and developmental processes leading to increase carcinogenesis (Eggen et al., 2010; Vilavert et al., 2012). Variations in the concentrations of the detected organic compounds in the various soil samples indicate that the constituents of the soil may depend on the type of oil spilled, the age and the metabolic activities inherent in the contaminated soil (Eggen et al., 2010; Vilavert et al., 2012) and these vary from one contaminated site to another.

Table 2. *Array of Metabolites Identified in Various Soil Samples Used in this Study*

Control	**6m-AB**	**12m-AB**	**18m-AB**
1,1'-Biphenyl, 2,4-dichloro-2',5'-dimethyl-	1-Heptyn-4-ol	1,3-Dioxolane, 2-(phenylmethyl)-	1-Ethenyl-3-(1-hexenyl)-4-trimethylsilylcyclopentane
1H-Pyrazole-3-carboxylic acid, 1-[(3-chlorophenyl)methyl]-, methyl ester	1-Hexen-4-ol, 1-chloro-3,5-dimethyl-	1,3-Dioxolane, 2-(1-phenylethyl)-	1-Hexen-4-ol, 1-chloro-3,5-dimethyl-
1-Naphthalenecarboxylic acid, 8-bromo-	1,3-Bis-t-butylperoxy-phthalan	1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-
1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether	1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	[2,6'-Bi-2H-1-benzopyran]-4(3H)-one, 3',4'-dihydro-3,5,7-trihydroxy-5'-methoxy-2',2'-dimethyl-, (2S-trans)-	2-Butanol, 3-methyl-
1,3-Bis-t-butylperoxy-phthalan	2-Butanone, 3-methoxy-3-methyl-	2-Butanol, 3-methyl-	2-Ethoxy-3-chlorobutane
1,3-Dioxolane, 2-(1-phenylethyl)-	2-Ethoxy-3-chlorobutane	2-Butanone, 3-methoxy-3-methyl-	2-Ethyl-3-ketovalerate, 2TMS derivative
1,3-Dioxolane, 2-(2-propenyl)-	2-Mercaptoethanol, TMS derivative	2-Ethoxy-3-chlorobutane	2-Vinylethyl acetate
1,3-Oxathiolane-4-carboxylic acid, 2-imino-5-phenyl-, ethyl ester	2-Vinylethyl acetate	2-Phenylisopropanol, TMS derivative	3,5-Dimethyl-5-hexen-3-ol
2-Propanamine, N-methyl-	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	2-Propanamine, N-methyl-	5-Methyl-4'-hydroxy-2-benzylidene-coumaran-3-one
2-Vinylethyl acetate	2,3-Dihydro-2-methyl-4-(4-methylphenyl)-1H-1,5-benzodiazepine	3-Hexene, 1-(1-ethoxyethoxy)-, (Z)-	9,9-Dichloro-9-silafluorene

Table 2. *Array of Metabolites Identified in Various Soil Samples Used in this Study (Continued)*

Control	6m-AB	12m-AB	18m-AB
2,3-Dichlorothiophene-5-sulfonyl chloride	2-[4-Chloro-trans-styryl]-4-chloropyrimidine	3-Pentanol, 3-methyl-	Benzenemethanol, _-[(methylamino)methyl]-
3-(1,2-Dibromoethyl)-1,1,2,2-tetrafluorocyclobutane	2,6-Dodecadienoic acid, 10-(bromoacetoxy)-11-methoxy-3,7,11-trimethyl-, methyl ester	3,4-Dimethyl-5-hexen-3-ol	Butane, 1-chloro-4-(1-ethoxyethoxy)-
3,3-Dichloropropyne	3-Buten-2-ol	3,6-Bis(trimethylsilyl)-1,4-cyclohexadiene	Methylarsine dibromide
3-Bromo-2-quinolinecarboxamide	3-Pentanol, 3-methyl-	4-Pyridinamine, 3,5-dibromo-	Methyl p-coumarate, TMS derivative
3-Methoxy-3-methylbutanol	3,4-Dimethyl-5-hexen-3-ol	5-(2-Methoxypropan-2-yl)-2-methyl-2-vinyltetrahydrofuran	Silane, (2-ethyl-4-methylene-1-cyclopenten-1-yl)trimethyl-
3-Pentanol, 3-methyl-	3,6-Bis(trimethylsilyl)-1,4-cyclohexadiene	5-Methyl-4'-hydroxy-2-benzylidene-coumaran-3-one	Silane, 9-anthracenyltrimethyl-
5-Methyl-4'-hydroxy-2-benzylidene-coumaran-3-one	4-Methoxy-4-methyl-2-pentanol	6,11-Dihydro-8-methoxy-1-benzopyrano[4,3-b] indole	Silane, trimethyl(1-phenylethyl)-
Benzyl alcohol, _-(1-(dimethylamino) ethyl)-	4-Pyridinamine, 3,5-dibromo-	9,9-Dichloro-9-silafluorene	Trifluoromethyltrimethyl silane
Methanol, chloro-, acetate	5-Methyl-4'-hydroxy-2-benzylidene-coumaran-3-one	Benzyl alcohol, _-(1-(dimethylamino)ethyl)-	
Methylarsine dibromide	5-Hexen-3-ol, 3-methyl-	Butane, 1-chloro-4-(1-ethoxyethoxy)-	
Methyl p-coumarate, TMS derivative	6,11-Dihydro-8-methoxy-1-benzopyrano[4,3-b] indole	Butanoic acid, 2-hydroxy-2-methyl-, methyl ester	
Propanoic acid, 3-chloro-	Bromo-dragonFLY	Methanol, chloro-, acetate	
Propane, 1,1-dimethoxy-	Butane, 1-chloro-4-(1-ethoxyethoxy)-	Methyl p-coumarate, TMS derivative	

Table 2. *Array of Metabolites Identified in Various Soil Samples Used in this Study (Continued)*

Control	6m-AB	12m-AB	18m-AB
Propane, 2-methoxy-2-methyl-	Butane, 2-methoxy-2,3,3-trimethyl-	Pyrimidine, 5-bromo-2,4-bis(methylthio)-	
Pyrimidine, 5-bromo-2,4-bis(methylthio)-	Methyl 10-(chloroacetoxy)-11-methoxy-3,7,11-trimethyl-2,6-dodecadienoate	Silane, (2-ethyl-3,3-dimethyl-4-methylene-1-cyclopenten-1-yl)trimethyl-	
Silane, 9-anthracenyltrimethyl-	Methyl p-coumarate, TMS derivative	Silane, 9-anthracenyltrimethyl-	
Silane, trimethyl(1-phenylethyl)-	Propanoic acid, 3-chloro-	Silane, tetramethyl-	
Trimethyl(3,3-difluoro-2-propenyl)silane	Propane, 2-methoxy-2-methyl-	Silane, trimethyl(1-phenylethyl)-	
Trifluoromethyltrimethylsilane	Pyrimidine, 2-(3,5-dimethylpyrazol-1-yl)-5-phenyl-	Spiro[(5-bromoacenaphthen-1-one)-2,2'-(5',5'-dimethyl-1',3'-dioxane)]	
	RS-2,3-hexanediol	(SR)- or (RS)-4-methyl-2,3-pentanediol	
	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	

Some of these contaminants usually bind to the soil particles and are slowly released into the environment (Schuhmacher et al., 1998; Lah et al., 2008).Heavy metals (nickel, cadmium, lead, and copper) were below detectable limits in bioremediated petroleum contaminated soil samples as well as in the control soil sample. This was unexpected since heavy metals cannot be degraded and some of them have been associated with petroleum and usually found at crude oil spill sites. Nonetheless, their non-detection in the reclaimed spill sites may be attributed to vertical and horizontal migration from top soil due to precipitation and flood events. The heavy metal content of the reclaimed soil samples was within the EGASPIN prescribed limits as shown in Table 3.

Table 3. *Heavy metal concentrations in bioremediated soil samples obtained from Ogoniland*

Heavy Metals	Sample Identity					
	Target Limit	**Intervention Limit**	**Control**	**6m-AB**	**12m-AB**	**18m-AB**
Nickel, Ni (mg/kg)	35	210	0.067	<0.001	<0.001	0.054
Copper, Cu (mg/kg)	36	190	0.035	0.016	<0.001	0.005
Lead, Pb (mg/kg)	85	530	0.04	0.11	0.07	0.05
Cadmium, Cd (mg/kg)	0.80	12	<0.001	<0.001	<0.001	<0.001

Conclusion

The TPH content of the bioremediated petroleum contaminated soil samples varied widely with that of pristine soil as TPH content was below detectable limits in the uncontaminated pristine soil whereas, in the bioremediated soils, TPH content was above the target value recommended by EGASPIN. However, PAH and heavy metals were below detectable limits in both uncontaminated and bioremediated soils. A wide array of metabolites 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 maybe deleterious to plant growth thus, suggesting the unhealthy status of the bioremediated soils for agricultural productivity. There is need to encourage further natural attenuation regime in bioremediated sites in order to ensure the obliteration of the residual contaminants and metabolic intermediates in soil before the return to agricultural activities on such sites.

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