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Biochemical and Physical Characterization of Diesel Petroleum Contaminated Soil in Southeastern Nigeria

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Abstract

The biochemical and physical characteristics of diesel oil contaminated soil were studied. The total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbon (PAH) concentrations were 46726.80 mg/kg and 844.40 mg/kg respectively for the diesel contaminated soil and 2.90 mg/kg and <0.001mg/kg for the control. The contamination increased the soil acidity, soil organic carbon, %Nitrogen and % silt to 5.9, 2.34 mg/kg, 0.202 mg/kg, 3.00 respectively compared to 6.7, 1.07 mg/kg, 0.092 mg/kg and 2.00 respectively in the control. The contamination also increased the concentration of heavy metals like Cadmium 0.25 mg/kg, iron 6311 mg/kg and lead 1.67 mg/kg against the uncontaminated soil with Cd 0.10 mg/kg, Cu 1.83 mg/kg, and Pb 1.22 mg/kg. Variation in individual concentrations of macro- and micronutrients in the contaminated and control soil samples was observed but did not affect the overall soil conductivity. The microbial growth rate expressed as colony forming units for bacteria and fungi were 1.3 x 10^9 cfu/g and 3.0 x 10^6 cfu/g respectively for the contaminated and 4.6 x 10^9 cfu/g and 4.0 x 10^6 cfu/g for the uncontaminated soil respectively. These changes created by diesel contamination resulted in the reduction in the pH and microbial biomass. These adverse changes could affect nutrient cycle, impede nutrient uptake by plant roots and subsequently lead to reduction in crop yield.

Keywords: Diesel-contamination, hydrocarbon, polyaromatic, nutrient, biomass, heavy metals.

Introduction

Diesel like all fossil fuels primarily consists of complex mixture of molecules called hydrocarbons. In large concentrations, petroleum products are highly toxic to many organisms, including humans[1]. The dominance of petroleum products in the world economy creates the condition for distributing large amounts of these toxins into populated areas and ecosystems around the globe[2].

The states in Nigeria in which oil has been found in considerable commercial quantities and also drilled are Rivers, Edo, Cross-Rivers, Imo, Abia, Akwa Ibom, Bayelsa and Delta State. Oils have also been struck in a few places in Anambra State[3].

With the continued utilization of diesel oil by many vehicles and generators, greater quantities of diesel oil are being transported over long distances. Therefore, diesel oil can enter into the environment through spillage. Factors that cause spillage range from those that affect the well head, to those that affect transportation to any of the various destinations as refineries, oil terminals or oil depots. In Nigeria, oil spillage may be as a result of oil pipe corrosion, pipeline/flow leakage, rupture of tanks, effluents from sabotage and human errors[4,5]. Since petroleum contains some gaseous components, these fractions will volatilize when there is oil pollution leaving the non-volatile components as residues in and on the soil [6]. It has been demonstrated that oil spillage affects the physical and chemical nature of soils[7]. Its components are known to be toxic to animals, plants and microbial life and the toxicity vary depending on the type of oil and additives used during refining and also on the biota of spillage[8]. In this research, we studied the biological, physical and chemical characteristics of a chronic diesel hydrocarbon contaminated soil.

Material and Methods

Sample Collection: The soil samples used in this study was collected from the diesel dump of the Federal university of technology Owerri. The soil in the sample area was chronically polluted with diesel. The top and sub-soil samples were collected with a sterile spatula into a sterile polyethylene bag, mixed and taken to the laboratory.

Microbial Characterization and Colony Count: Microbiological methods for the identification and enumeration of bacteria and fungi isolates were conducted aseptically[9,10]. Microorganisms in the diesel contaminated and uncontaminated soils were enumerated by spread inoculating 0.1ml of serially diluted sample onto nutrient agar (NA) plates for the enumeration of aerobic heterotrophic bacteria. Bacterial organisms were subjected to biochemical tests (such as oxidase, catalase, citrate, indole test) and identified using Bergey's manual of determinative bacteriology. Fungi were identified on Sabourand dextrose agar, subjected to microscopy and morphology identification and then compared with those established.

Determination of Total Petroleum Hydrocarbon (TPH) and Polyaromatic Hydrocarbon (PAH) by Gas Chromatograph: The soil samples were cleaned of roots, thoroughly mixed, 2 g of each soil sample was weighed into a clean extraction container. Then 10 ml of extraction solvent (pentane) was added into each sample, mixed thoroughly and allowed to settle. The mixtures were carefully filtered into a clean solvent extraction vessel, using filter paper fitted into buchner funnels. The extracts were concentrated to 2 ml and then transferred for cleanup/separation. The concentrated aliphatic fractions were transferred into labeled vials with teflon caps for gas chromatograph (GC) analysis. The TPH and PAH were measured following USEPA Method 8015B for GC analysis with a FID detector, and a HP-5 column was used. External calibration was conducted with the original diesel fuel.

Determination of Physicochemical properties of Soil: The pH of the soil samples were measured by glass electrode pH meter in 1:1 water to soil ratio, estimated electrometrically[11]. Electric conductivity was estimated with Conductivity meter; cation exchange capacity (CEC) by NH_4 saturation, organic carbon, nitrogen, sodium, potassium and calcium were determined according to the methods of AOAC[12]. Effective CEC was calculated by the sum of exchangeable bases (Ca, Mg, K, and Na) and exchangeable Al and H expressed in meq/100g. Heavy metals (Cd, Pb, Cu, Zn, Mn, and Fe) were estimated analyticaly[13] after ashing with atomic absorption spectrophotometer (Perkin-Elmer Model 403). Hydrometeric method[14] was used for soil mechanical determination.

Results and Discussion

The volume of crude oils or petroleum products used today surpasses all other chemicals of environmental and health concern. Due to the number of facilities, individuals and processes and the various ways the products are stored and handled, environmental contamination is potentially widespread. In this study the total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbon (PAH) were 46726.80 mg/kg and 844.40 mg/kg respectively for diesel contaminated and 2.90 mg/kg and <0.001mg/kg for uncontaminated soil. The high TPH and PAH are as a result of diesel oil contamination. These concentrations of the TPH and PAH can make soil conditions unsatisfactory for plants and microbial growth[15]. Studies have shown that PAHs can be carcinogenic and/or mutagenic in some circumstances and have been classified as priority pollutants[16]. This concentration of contamination will also increase the presence of toxic materials such as cresol, phenols, chlorine which may inhibit the growth of the hydrocarbon oxidizers.

The contamination may have resulted in the low pH value of 4.70 observed in the contaminated soil compared to the 6.70 value observed in the uncontaminated. The low pH may have affected the microbial growth in the contaminated soil, which was observed to be low. A study have found optimal activity for microbial degradation at a pH of 7.4 and considerable inhibition at pH 4.5 and 8.5[17]. The optimum pH for best performance of most tropical crops is 6-7 when most essential nutrients are available at adequate amounts[18]. Similarly the high content of PAH may have also caused reduced microbial biomass observed in contaminated soil. PAHs are relatively persistent and recalcitrant in soils and are usually difficult to be degraded under natural conditions[19].

Table-1 and table -2 showed the bacteria- *Bacillus sp, Corynbacterium sp, Micro coccus, Staphycoccus sp* were isolated from the uncontaminated soil while *Bacillus subtilus and Micrococcus* were isolated from diesel oil contaminate soil also fungi such as *Aspergillus sp, Mucor sp, Rhizopus sp, Penicillin sp, Rhizopus sp*, were isolated from the uncontaminated soil while *Aspergillus sp, Rhizopus sp and Fusarium sp* were isolated from diesel oil contaminate soil. The loss in the microbial specie and population in the diesel contaminated soil could be as a result of the high concentration of the diesel oil especially the PAHs which are highly toxic to microbial cell membranes

The results on the bacterial growth rate expressed in bacterial colony forming units were 1.3 x 10^9 cfu/g in the contaminated and 4.6 x 10^9 cfu/g in the uncontaminated soil. The fungal growth rate expressed in colony forming units were 3.0 x 10^6 cfu/g in the contaminated and 4.0 x 10^6 cfu/g in the uncontaminated soil. The presence of $C_5 - C_{10}$ homologues in the petroleum fraction have been shown to be inhibitory to the majority of hydrocarbon degraders[20]. As solvents, these homologues tend to disrupt lipid membrane structures of microorganisms. The lower microbial population in the diesel contaminated soil could be as a result of the effect of the diesel oil which could lead to impairment of gaseous exchange and retention of soil carbon dioxide. This condition results in increased acidity and decreased porosity of the soil. The immediate effect of diesel oil contamination in the soil is the depression of the microbial population due to the presence of additives in the refined diesel oil.

Table - 1
Biochemical Characterization of Bacteria Isolates in Contaminated and Uncontaminated Soil

Colony code	Gram reaction	Microscopic morphology	catalase	citrate	oxidase	Indole	uncontaminated	contaminated
							Most probable identity	Most probable identity
Cream irregular	Gram positive	Cocci with short chain	+ve	+ve	-ve	-ve	*Bacillus sp*	*Bacillus subtilus*
orange	Gram positive	Rod shape	+ve	+ve	-ve	-ve	*Corynbacterium sp*	*No isolate*
yellow	Gram positive	Cocci with chister tetras	+ve	+ve	-ve	-ve	*Micrococcus*	*Micrococcus*
Golden yellow	Gram positive	Cocci with chister	+ve	+ve	-ve	-ve	*Staphylococcus sp*	*No isolate*

Table - 2
Microscopic Appearance of Fungi Isolates from Uncontaminated and Contaminated Soil Sample

Colonial Description	Microscopic Appearance	Uncontaminated	Contaminated
		Most probable identity	Most probable identity
Black spores [85mm] white periphery	Septate hyphae condia lobased and formet on stemfoma	*Aspergillus sp*	*Aspergillus sp*
White flat colours	Non septate hyphae	*Mucor sp*	*No isolate*
White colony hyphae	Non septate hyphae spore inside sporogiosprce	*Rhizopus sp*	*Rhizopus sp*
Green colonies with white periphery	-	*Penicillin sp*	*No isolate*
White cutton hyphae	-	*Rhizopus sp*	*No isolate*
Thick whitish colonies	-	*Geotridum sp*	*No isolate*
Greenish spores with white periphery	Septate hyphae	*Fusarium sp*	*Fusarium sp*

Table - 3
Total Colony Count, TPH and PAH in Uncontaminated and Contaminated Soils

Sample	bacteria (cfu/g)	fungi (cfu/g)	TPH (mg/kg)	PAH (mg/kg)
Uncontaminated soil	4.6×10^9	4.0×10^6	2.90	<0.001
Contaminated soil	1.3×10^9	3.0×10^6	46726.80	844.40

Table - 4
Physicochemical properties of contaminated and uncontaminated soils

Parameters	Uncontaminated Soil	Contaminated Soil
% Sand	85.74	81.74
% Silt	2.00	3.00
% Clay	12.26	15.26
Texture Class	Loamy Sandy	Loamy Sandy
Organic Carbon (mg/kg)	1.07	2.34
% Nitrogen (mg/Kg)	0.092	0.202
pH	6.7	5.9

Table - 5
Trace mineral content of uncontaminated and contaminated soils

Parameters	Uncontaminated Soil	Contaminated Soil
Na (mg/kg)	31.70	31.20
Ca (mg/kg)	28.10	9.60
K (mg/kg)	23.90	21.60
Mg (mg/kg)	138.50	51.00
Conductivity (µS/cm)	30	10
Na(mEq/100g)	0.138	0.136
Ca(mEq/100g)	0.140	0.048
K(mEq/100g)	0.061	0.055
Mg(mEq/100g)	1.154	0.425
Exchangeable Al(mEq/100g)	0.52	0.27
Exchangeable H(mEq/100g)	1.67	1.17
CEC(mEq/100g)	3.683	2.104

Table – 6
Heavy metals content of uncontaminated and contaminated soils

Parameters	Uncontaminated Soil	Contaminated Soil
Cadmium(mg/kg)	0.10	0.25
Copper(mg/kg)	1.83	1.30
lead(mg/kg)	1.22	1.67
Iron(mg/kg)	4948	6311
Zinc(mg/kg)	117.4	3.21

The additives and other modifying chemicals could prevent rapid microbial utilization of the hydrocarbon in the diesel oil. The loss of these microorganisms reduces the biological activity in the contaminated soil. Our result in table 4 showed higher percentage value of nitrogen - 0.202 mg/kg in the uncontaminated soil and 0.092 mg/kg in the contaminated soil. This increase could come from the nitrogen content of the refined diesel[21,22].

The reduced ability of the microbial population in the contaminated soil to adequately utilize the available nitrogen present in the soil before contamination is an important factor as well. Similarly, PAHs are hydrophobic compounds with low solubility in water, which have a greater tendency to bind with organic matter or soil, limiting their availability to microorganisms[23,24]. The results showed that the organic carbon value of 2.34 mg/kg in the diesel oil contaminated soil was higher than 1.07mg/kg value in uncontaminated soil. This was probably due to the effect of contamination with hydrocarbons in the soil[25]. The higher percentage silt (3%) and clay (15.26%) in contaminated soil suggests a reduction in the soil aeration and porosity, these conditions can affect crop yield[26].

The results in table 5 show a reduction in the trace mineral content of the contaminated soil. This reduction may have caused the lower conductivity value of 10 µS/cm in the contaminated soil when compared to 30 µS/cm in the uncontaminated. Soil conductivity is a measure of the soluble salts content in the soil and is used as an overall indicator of the level of macro- and micronutrients in the soil. This indicates that the diesel contaminant disturbed the soil structure and modified its physicochemical properties[27,28]. The reduction in the concentrations of sodium, calcium, potassium and magnesium which are suitable terminal electron acceptor[32] will affect the indigenous microbial metabolism. The cation exchange capacity (CEC) value of 3.683 Meq/100g was higher in the uncontaminated soil compared to 2.104 Meq/100g in contaminated. The CEC values suggest that the uncontaminated soil have higher quantity of cations that can be adsorbed and held by soil and therefore available for plant use. The higher CEC value also indicates a better pH buffering capacity.

The presence of heavy metals in the environment and specifically, in soils, industrial and domestic urban wastes endangers living organisms. Once it gets into food chain, through plants, animals and water sources leads to biomagnification and bioaccumulation in living cells and tisues[29,30].

Conclusion

The results obtained in this study revealed that diesel at high concentrations altered the microbial, physical and chemical properties of the soil. It affects soils' structural class and as well, to increased acidity which can affect plant roots and subsequently impede nutrient uptake from the soil. This finally may lead to reduction in crop yield.

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