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COMPARATIVE EFFECTS OF *Arachis hypogaea* and *Sorghum bicolor* ON TOTAL PETROLEUM AND PHYSICOCHEMICAL PROPERTIES OF CRUDE OIL CONTAMINATED SOILS

***IHEME, P.O., AKINOLA, M.O. AND NJOKU, K.L.**
Department of Cell Biology and Genetics University of Lagos, Akoka, Nigeria
*Corresponding author: Odichi2001@yahoo.com

Abstract

This study compared the effects of Arachis hypogaea and Sorghum bicolor on the total petroleum hydrocarbon (TPH) content, pH, moisture content, total organic matter, total organic carbon, total nitrogen and available chloride, sulphate and phosphorus ions of crude oil contaminated soil. Pot experiments were conducted in which A. hypogaea and S. bicolor were grown in soil artificially contaminated with different concentrations of crude oil (0%, 1%, 2%, 3% w/w). All treatments were replicated three times. The soil parameters were determined by following standard methods while the TPH and hydrocarbon fractions were determined using the Gas chromatography techniques. The result showed that during the period of study the pH of the soil increased significantly (P≤0.05) in this order; soil without plant > soil with S. bicolor> soil with A. hypogaea in all crude oil contaminations except in 2%. All other soil properties including the TPH content significantly reduced (P≤0.05) in the same order in all crude oil contamination levels. The lower TPH content of soil with A. hypogaea observed in this study suggests that its growth has improved soil dynamics and working capacity than S. bicolor. This may be a pointer that A. hypogaea will do better than S. bicolor in reclamation of crude oil contaminated soil.

Key Words: *Crude oil, contamination, reclamation, Arachis hypogaea, Sorghum bicolour*

Introduction

Since the mid-1950s crude oil became the world's most important source of energy and raw materials for chemical products (Harold, 2014) and has accrued high global economic and political value. This has led to expansion of the oil industry as well as the problems associated with crude oil exploration, processing, transportation and usage. One of such problems is oil spillage on the seas and lands which has become a recurrent global problem and cannot be overlooked (Sonawdekar, 2012). Studies on the effects of crude oil on soil properties have shown that crude oil pollution increases soil C/N ratio and induces limitation in soil nitrogen and phosphorus (Obasi *et al.*, 2013). Crude oil spillage causes inhomogeneous spread of water in soils leading to water deficiency in some parts and water logging in others (Nwaoguikpe, 2011) and increases soil bulk density (Oyem, 2013). It prevents oxygen exchange and sufficient aeration (Onuoha *et al*., 2003), causes low soil pH thereby leading to absorption of some heavy metals up to toxic levels (Essiet *et al*., 2010)and increases soil salinity (Oyem, 2013).

Several physical and chemical processes have been applied so as to reclaim the environment but biological processes are preferred due to the expensive nature and inadequacies of the physical and chemical processes. However, the need for a more cost effective, environmentally friendly and efficient method gave way to phytoremediation. It is defined as the use of plants for *in situ* removal or reduction of pollutants from polluted media (Gianfreda *et al.,* 2010). This depends on the extraordinary metabolic and extractive capabilities of plants Pilon-Smith (2005).Huang *et al*. (2004) suggested that plants enzymatic set, extensive root system and interactions between the plant and microbes within its rhizosphere are keys to accelerated remediation kinetics.

This study compared the effects of *Arachis hypogaea* and *S. bicolor* on the total petroleum hydrocarbon (TPH) content, pH, moisture content, total organic matter, total organic carbon, total nitrogen and available chloride, sulphate and phosphorus ions of crude oil contaminated soil.The selected plants for this study have been reported to grow and tolerate crude oil contaminated soil. For instance *S. bicolor* has been reported to grow in oil contaminated fields of Iran (Shirdam *et al*. 2008). These plants have high biomass, extensive root system and have the ability to thrive on contaminated soil. In addition *A. hypogaea* with the aid of bacteria habouring its root nodules can fix nitrogen to improve soil conditions in the presence of contaminants while *S. bicolor* can tolerate low soil fertility, water logging and high salinity that characterize crude oil contaminated soil (Schwab *et al*., 2006).

Materials and Methods

Collection of Materials: The crude oil was obtained from Exxon Mobil offshore facility (Erha light crude). The seeds of *A. hypogaea* and *S. bicolor* were collected from the gene bank of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria. Sandy loam soil used in this study was collected from the uncultivated rain forests within the University of Lagos environment at a depth of 0-15cm (Salami and Elum, 2010).

Preparation of Soil: The soil was air-dried at 28^0C and passed through a 5mm sieve to remove stones and debris for easy absorption of crude oil (Njoku *et al*., 2012). A grain analysis was done according to the method specified by the British Standard Institution (BSI) for soil tests for civil engineering purposes, BS 1337: Part 2: (1990) using the wet/dry sieve methods. Conditions of major oil spill were simulated by adding different amounts of crude oil (100g, 200g and 300g) on 10kg sandy loam soil in experimental pots by spraying and continuous mixing to get 1%, 2%, 3%, levels of contamination.

Planting

The modified method of Eze *et al.* (2013) was used in which eight seeds of groundnut and sorghum were sown separately into the crude oil contaminated soil in the containers of depth 10cm and circumference 100cm respectively. The groundnut seeds were planted at 2cm depth while the sorghum seeds were planted at 5cm depth.

Sampling and Analyses: This soil sample was collected at the beginning and at the end (120 days) of the study to analyze for soil physicochemical properties and TPH. The pH of the soil was determined by using the method described by Eckert (1995) using 1:1 soil to water ratio. Soil moisture was determined by the method described by Schneekloth *et al*. (2002) by weighing soil dried at 105±5^{0}C for 24 hours and was calculated from the difference in weight. Soil total organic matter content and organic carbon content was determined as described by Onifade *et al*. (2007). Total organic carbon was determined by acid digestion and titration methods and total organic matter was calculated from the value of total organic carbon multiplied by conversion factor. Total phosphorus and available sulphate was determined spectrophotometrically as described by Ben Mussa *et al*. (2009). Total nitrogen was determined by using the micro-Kjeldahl method as described by Jones *et al*. (1991). The available chloride content was determined by the amount of silver nitrate consumed in the titration (Texas Department of Transportation, 2005). Total petroleum hydrocarbon was determined by EPA method 8015B with modification as cited by Wenhao *et al*. (2007).

Statistical Analysis

The data obtained were analysed with SPSS version 23 software using ANOVA and t test followed by Duncan's multiple range tests at 5% level of significance.

Results

Table 1 shows the effect of plant growth on soil properties of contaminated soil. There was a general decrease in the soil properties from initial to all final treatments in this order: initial soil> final soil without plant> soil with *S. bicolor*> soil with *A. hypogaea* except the pH that increased. The pH of the soil without plant was higher with a level of significance (P≤0.05) than the pH of the soil with plants except at 2% crude oil contamination. However, pH in soil with *S. bicolor* was higher than in *A. hypogaea* with a level of significance (P≤0.05) in 1% and 3% crude oil contamination.

At the end of the study there was a significant decrease (P≤0.05) in total organic matter content and total organic carbon content of all final soil treatments when compared with the initial within the crude oil contamination levels and as crude oil contamination increased. The least values observed were (35.53±0.01%) for total organic matter and (3.48±0.02%) for total organic carbon in *A. hypogaea* soil with 3% crude oil contamination. Total organic matter and total carbon content of the soil with *S. bicolor* was significantly higher (p≤0.05) than that of soil with *A. hypogaea* in all the levels of crude oil contamination. Available chloride ion, sulphate ion, phosphorus ion and total nitrogen in the initial contaminated soils were significantly higher than the final without plant and with plant and as crude oil contamination increased with the least values for all the parameters observed in *A. hypogaea* 3% crude oil contamination. Available chloride ion, sulphate ion, phosphorus ion and total nitrogen of soil with *S. bicolor* were significantly higher (P.≤0.05) than those of *A. hypogaea* in all the crude oil contamination levels.

Table 1: Physicochemical properties of crude oil contaminated soil with *A. hypogaea* and *S. bicolor*

Conc.	Soil treatment	pH	Moisture	TOM	TOC	Available chloride	SO_4^{2-}	Total Nitrogen	P
1%	initial	5.84±0.02^{c}	7.19±0.01^{a}	84.96±0.01^{a}	13.70±0.02^{a}	8.79±0.01^{a}	74.78±0.03^{a}	64.92±0.02^{a}	35.85±0.01^{a}
	No plant (final)	6.24±0.03^{a}	4.90±0.11^{b}	42.89±0.06^{b}	5.15±0.05^{b}	2.56±0.04^{b}	35.18±0.02^{b}	20.04±0.04^{b}	11.76±0.01^{b}
	S. bicolor (final)	6.14±0.14^{b}	4.35±0.02^{c}	38.40±0.01^{c}	4.15±0.01^{c}	2.16±0.01^{c}	30.85±0.03^{c}	13.21±0.02^{c}	6.91±0.01^{c}
	A. hypogaea (final)	5.99±0.01^{bc}	3.93±0.03^{d}	35.99±0.01^{d}	3.68±0.01^{d}	1.95±0.02^{d}	29.10±0.07^{d}	10.09±0.08^{d}	5.97±0.01^{d}
2%	Initial	5.61±0.03^{b}	7.71±0.03^{a}	84.54±0.04^{a}	13.24±0.04^{a}	8.71±0.01^{a}	74.25±0.25^{a}	64.59±0.02^{a}	35.38±0.02^{a}
	No plant (final)	6.18±0.01^{a}	4.75±0.05^{b}	42.85±0.03^{b}	4.94±0.01^{b}	2.30±0.03^{b}	34.61±0.02^{b}	18.60±0.06^{b}	9.90±0.03^{b}
	S. bicolor (final)	6.09±0.09^{a}	4.15±0.03^{c}	38.35±0.01^{c}	3.99±0.01^{c}	2.09±0.04^{c}	30.15±0.01^{c}	12.98±0.01^{c}	6.88±0.01^{c}
	A. hypogaea (final)	6.02±0.07^{a}	3.92±0.02^{d}	35.55±0.01^{d}	3.56±0.02^{d}	1.90±0.02^{d}	27.06±0.06^{d}	10.09±0.01^{d}	5.82±0.01^{d}
3%	Initial	5.25±0.07^{c}	8.10±0.04^{a}	84.16±0.01^{a}	13.19±0.02^{a}	8.65±0.03^{a}	74.05±0.10^{a}	64.30±0.03^{a}	35.13±0.04^{a}
	No plant (final)	6.16±0.02^{a}	4.50±0.02^{b}	42.15±0.05^{b}	4.61±0.03^{b}	2.28±0.03^{b}	33.19±0.04^{b}	18.25±0.05^{b}	9.61±0.03^{b}
	S. bicolor (final)	6.12±0.01b^{a}	4.15±0.05^{c}	38.10±0.02^{c}	3.97±0.04^{c}	2.07±0.01^{c}	29.26±0.02^{c}	12.88±0.04^{c}	6.17±0.02^{c}
	A. hypogaea (final)	5.96±0.00^{b}	3.90±0.03^{d}	35.53±0.01^{d}	3.48±0.02^{d}	1.66±0.03^{d}	27.00±0.01^{d}	10.07±0.02^{d}	5.78±0.07^{d}

Column value represent mean±Std. dev. (n=3) follow by the same letters are not significant at $P > 0.05$ by DMRT per crude oil concentration treatment; TOM: total organic matter; TOC: total organic carbon; P: available phosphorus ion

Table 2: TPH content of crude oil contaminated soil with *A. hypogaea* and *S. bicolor*

Trt	Initial TPH	Final TPH in soil without plant	Final TPH in soil with *A. hypogaea*	Final TPH in soil with *S. bicolor*	Percentage remediation by *A. hypogaea*	Percentage remediation by *S.bicolor*
1%	2868.88	155.05±0.02	80.72±0.01	104.78±0.01	47.94b	32.42a
2%	3156.72	133.06±0.01	71.22±0.01	90.49±0.01	46.47b	31.99a
3%	3353.89	120.33±0.04	70.10±0.00	84.43±0.01	41.74b	29.83a

Values represent mean±SD (n=3) followed by the same letter are not significantly different at $P \geq 0.05$;

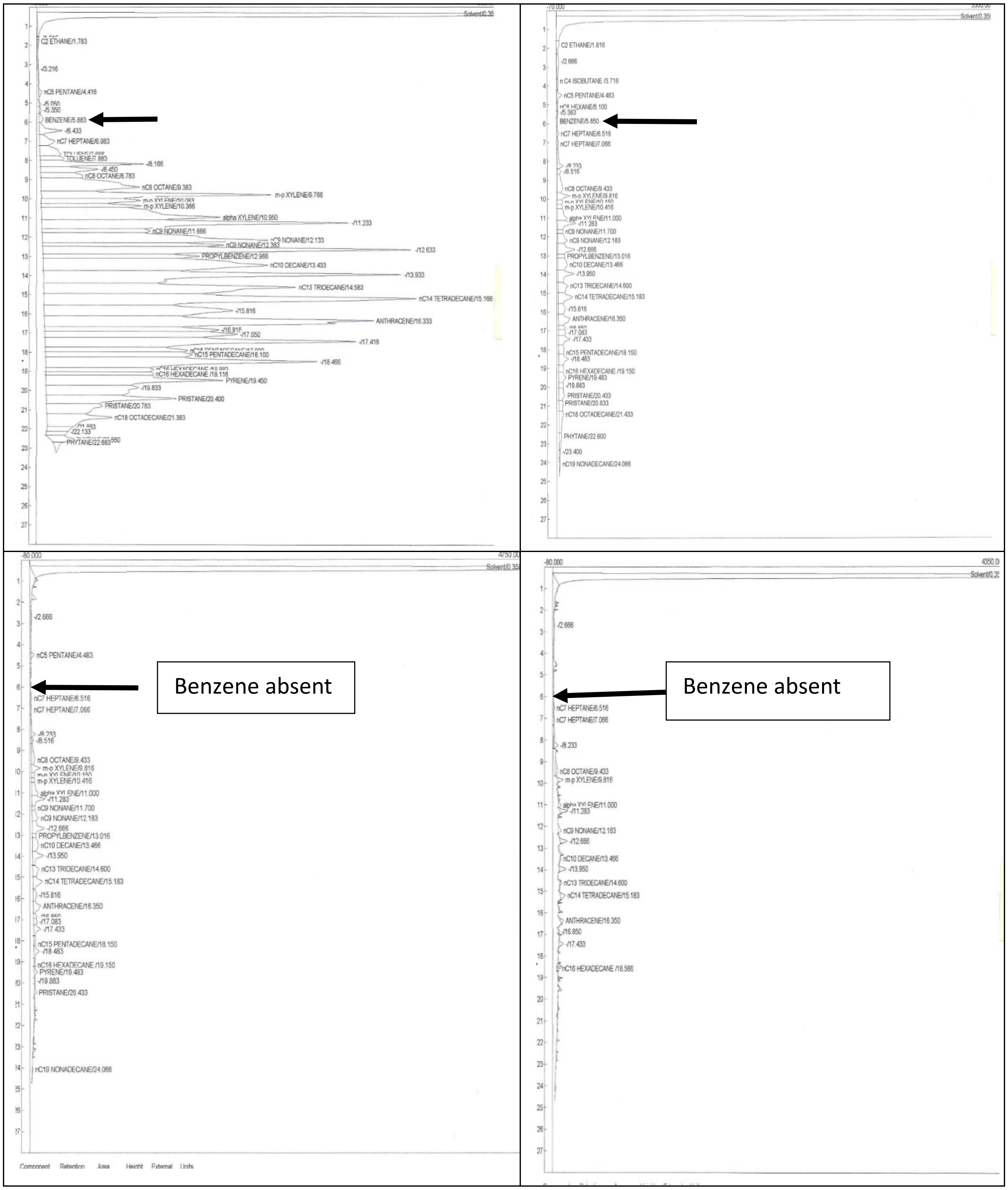

Figure 1: Chromatographs showing hydrocarbons from (a) initial soil without plant (b) final soil without plant(c) Final soil with *A. hypogaea* (d) Final soil with *S. bicolor*. Arrows pointing at Benzene location on the chromatographs

There was a decrease of TPH content for all soil treatments as the level of crude oil contamination increased. Highest TPH level (155.05±0.02 mg/kg) was observed in final soil without plant at 1% contamination while the least (70.10±0.00mg/kg) was in soil with *A. hypogaea* at 3% level of crude oil contamination. Percentage loss (remediation) due to *A. hypogaea* was significantly higher than that due to *S. bicolor* ($P \leq 0.05$) in all the levels of crude oil contamination.

Hydrocarbon fractions showed significant difference ($P \leq 0.05$) between the treatment groups in this order: initial> final without plant>soil with *S. bicolor*> soil with *A. hypogaea* except for isobutene, hexane, toluene and nonadecane which were absent in the initial but observed after 120 days in soil without plant, soil with *A. hypogaea* and soil with *S. bicolor*. Benzene which was present in initial and final soil without plant was absent in soils with plants (figure 1).

Discussion

The result on the effect of *A. hypogaea* and *S. bicolor* on pH of crude oil contaminated soil is in accordance with the report of Osuji and Nwoye (2007), Essiet *et al*. (2010) and Obasi *et al.* (2013) who posited that low pH or acidity of crude oil contaminated soil could be as a result of the free cations present in crude oil, causing them to have properties of weak acids or because crude oil discourages the leaching of basic salts which are responsible for increased pH as found in non-oil contaminated soil. The result showed that natural attenuation and the growth of plants have buffering effects on crude oil contaminated soil. The higher pH content of contaminated soil with *A. hypogaea* and *S.bicolor* conforms with the report of Njoku *et al.* (2009) who reported that *Glycine max* caused an increase in pH of crude oil contaminated soil and Njoku *et al*. (2014) who reported that *Eleusine indica* and *Pennisetum glaucum* led to a significant increase in pH of crude oil contaminated soil when compared to soil without plant. The lower pH of contaminated soil with *A. hypogaea* when compared to soil with *S. bicolor* may be as a result of increased microbial activity due to decay of fallen leaves which was absent in soil with *S. bicolor* or its nitrogen fixing ability. Osuji and Nwoye (2007) explained that microbial metabolism leads to production of organic acids that reduce pH in contaminated soil.

The reduction of the moisture content of all final soil treatments compared to the initial in all crude oil contaminations could be as a result of natural attenuation and the effect of plant growth. The reduction of the moisture content of the planted soils may be because plant roots created pore in the soil and enhanced water penetration and infiltration. This conforms to the findings of Njoku *et al.* (2009) who reported decreased moisture content in 25g crude oil contaminated soil with G. max after 42 days. The lower moisture content in contaminated soil with *A. hypogaea* may be that there was more microbial activities which could enhance soil porosity and more water penetration and infiltration than *S.bicolor* soil. Also the activities of nitrogen fixing bacteria in the root nodules of *A.hypogaea* suppress the effect of nutrient deficiency which is characteristic of crude oil contaminated soils. This encouraged luxuriant root growth of

A.hypogaea, more water penetration and infiltration which may have resulted to low moisture content observed.

The reduction of total organic matter and total organic carbon in all soil treatment after 120 days could be because of natural attenuation and the growth of the plants. This result conforms to the findings of and Njoku *et al.* (2014) who reported low organic matter content of the soils planted with *Panicum maximum, Eleusine indica* and *Pennisetum glaucum* when compared to soils without plant. Osuji and Nwoye (2007) also reported that reduced organic matter in crude oil contaminated soil is as a result of the impairment of metabolic process by crude oil thereby reducing addition of carbon mineralization and capacity of the microflora. Then with the introduction of plants, rhizosphere effect may possibly lead to further utilization of the total organic matter and carbon for energy leading to their reduction in the soil after 120 days. The higher total organic matter in contaminated soil with S. *bicolor* when compared with A. *hypogaea* could possibly be because of low microbial activity and reduced usage of total organic matter and carbon. Total organic matter improves the binding process in the soil. According to Njoku *et al.* (2009), binding increases water retention capacity of the soil and this may also be the reason for the higher moisture content of contaminated soil with *S. bicolor* than soil with *A. hypogaea*.

Total nitrogen, available chloride ion, sulphate ion, and phosphorus ion of all final soil treatments reduced significantly when compared to the soil before remediation (initial). The mentioned parameters are higher with a level of significance ($P< 0.05$) in *S. bicolor* treated soil when compared to *A. hypogaea* treated soil. The reason for diminution of these properties in *A. hypogaea* could be that more activities took place in soil with *A. hypogaea* possibly due to its leguminous qualities and low phytate content that increases its ability to absorb nutrients faster from the soil than *S. bicolor*.

The soils with plants lost more TPH than the soils without plant. This is similar to the result obtained by Rosado and Pitchel (2004), Merkl (2005b), Shirdam *et al.* (2008) and Njoku *et al*. (2009), who reported loss of TPH in vegetated soils than in unvegetated soils. According to the authors, these observations can possibly be linked to one or the several techniques of phytoremediation such as increased microbial activity that is attributable to root exudates and oxygen input from roots. Growth of *A. hypogaea* led to a greater loss of TPH from the soil than S *bicolor* in all the crude oil contamination level showing that *A. hypogaea* has a greater efficiency in remediating crude oil contaminated soil than S *bicolor.* Yateem *et al.* (2000) got a similar result in which alfalfa (*Medicago sativa*) and broad bean (*Vicia faba*) led to a greater loss of TPH from the soil than rye grass (*Lolium perenne*). Hutchison, (2001) reported greater decontamination of TPH by *Cynodon dactylon* (68%) than Festuca (62%). The report of Diab (2008) corresponds to the finding above in which TPH loss was enhanced in the rhizosphere of a legume *Vicia faba* to the tone of 30% as against 16% and 13.7% in *Zea mays* and *Triticum aestivum* . The greater efficiency of *A. hypogea* than *S. bicolor* in removing crude oil from the soil could be as a result of difference in metabolic functions of the plants and composition of root exudates and therefore by their distinct impact on microbial activity. Plant metabolic functions such as nitrogen fixation in the soil by *A.hypogea* greatly reduced plant microbe competition for nitrogen, increasing plant root exudates production which attracts more microbial population and diversity. This conforms to the report of Ndimele (2010) that legumes are good options for phytoremediation of petroleum hydrocarbons.

The chromatogram of the crude oil in initial soil show higher concentration of hydrocarbon fractions than in final soils with or without plants. This can be attributed to the degrading effect of enzymes from root exudates and plant associated microbes that have overcome the effect of the crude oil with time and (natural attenuation) and the growth of plants. This result conforms to that of Aprill and Sims (1990) in which the extent of disappearance of PAH was consistently greater in planted units than unplanted controls. Lower concentrations of hydrocarbon fractions and total loss of C_2ethane, Isobutane, $_nC_6$hexane, $_nC_{18}$ Octadecane and phytane found in *A. hypogaea* soils compared to *S. bicolor* soil indicate that *A. hypogaea* had better performance in degradation of crude oil than *S. bicolor*. Qui *et al*. (1997) got similar result in which Verde Klein grass, accelerated the loss of high molecular weight pyrene, benzo(a)anthracene and benzo(a)pyrene compared to other grasses. This may be because *A. hypogaea* habours more microbes or has the specific microbes for the degradation of the mentioned fractions than *S.bicolor*. According to Thapa *et al.* (2007) different species of microbes preferentially attack different compounds leading to a total loss of one compound or the other as observed in this study. The total loss of $_nC_{19}$nonadecane in *S. bicolor* soil can also be explained as above. The microbial action is stimulated by root exudates, other favourable soil conditions introduced by the plant roots which may have been better in contaminated soil with *A. hypogaea* due to its leguminous nature.

Conclusion

The result of this study shows that the growth of *A. hypogaea* and *S. bicolor* affected the physic-chemical properties of the contaminated soil and enhanced crude oil remediation. However *A. hypogaea* showed higher remediation efficiency than *S. bicolor* with soil properties more exhausted. This calls for the use of soil augmentations like animal manure to improve soil conditions during remediation of crude oil.

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