ASSESSING SUCCESSIVE PLANT GROWTH ON PETROLEUM HYDROCARBON DEGRADATION IN HIGHLY POLLUTED SOIL AUGMENTED WITH WOOD ASH

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Abstract

Successive growth of Corchorus aestuans L. and Lagenaria siceraria (Mol.) Standl. in crude oil polluted soil supplemented with wood ash were evaluated for degradation and compositional changes of crude oil components in soil for 5.5 months. The vegetative parameters recorded were germination, plant height or vine length and leaf elongation rate. Total petroleum of hydrocarbon (TPH) and pH of the soil samples (unpolluted and polluted) obtained from the field were determined before the commencement of study. GC – FID analyses of differently prepared soil samples obtained at the end of the experiments were carried out as well. Mean values obtained for TPH and pH of unpolluted and polluted soil from the field were 10.90 ppm and 40,173.90 ppm, 6.26 and 4.67, respectively. The application of wood ash as soil supplements in crude oil polluted soil changed the hydrophobic soil condition to hydrophilic. This also improves the pH toward neutral. Germination was observed in all concentrations of soil tested. Percent germination of C. aestuans was 40% and below while L. siceraria was higher. Over 90% of C. aestuans seedlings could not survive up to 60 days in polluted soil. Growth inhibition in polluted soil was recorded. The growth of L. siceraria plants on the same soil was also inhibited but they sustained growth for a longer period. Minimal plant growth enhancement was observed for plants grown in 50% polluted soil supplemented with 20g wood ash. The GC-FID chromatograms showed presence of aliphatic and aromatic hydrocarbons at high concentration in polluted soil obtained from the field. These petroleum hydrocarbon compounds were degraded in soil following wood ash supplementation and plant growth. The chromatograms showed the absence of some crude oil components after plant growth, and where present but at very insignificant concentrations detected. Interestingly, both aliphatic and aromatic compounds were degraded following the wood ash supplementation in polluted soil. In contrast, some other studies have reported selective degradation of hydrocarbons in contaminated soil. The challenge in applying accelerated biodegradation techniques is not the ability to stimulate bacteria to degrade hydrocarbons; it is judging how low the hydrocarbon concentration can be.

Keyword: polluted soil, *Corchorus aestuans, Lagenaria siceraria,* TPH

INTRODUCTION

Crude oil (hydrocarbon) pollution generated from exploration and processing of petroleum is a widespread environmental problem. Crude oil contains numerous components including polar hydrocarbons, n-alkanes, unresolved complexes of branched- and cyclo-alkanes as well as aromatics, resin- and asphaltene residuals (Killops and Al-Jaboori, 1990; Outdot *et al*., 1998). The environmental risk predominantly derives from suspected or proven mutagenic properties of polycyclic aromatic hydrocarbons (PAHs) and their resistance toward degradation (WHO, 1983); finally affecting soil use and fertility. Any oil spill would usually damage soil properties and plant communities due to the associated changes in soil conditions, e.g. when nutrients or elements essential for plant growth are made unavailable (Anoliefo *et al*., 2003) and dehydration. Phytoremediation involves the use of plants to stabilize hydrocarbon polluted soils (re-vegetation) and to enhance hydrocarbon degradation by stimulating soil microbes/microbial consortia with hydrocarbon degradation capabilities in rhizosphere (Wenzel, 2009). Microbial degradation of oil products is a principal process in the elimination of petroleum from the environment (Zobell, 1964). Bacteria and fungi have the capacity to degrade a wide range of oil components, which exist throughout the ecosystem. There are basically two main approaches to oil bioremediation.

1) Bioaugmentation: The addition of oil-degrading bacteria to supplement the existing microbial population;
2) Biostimulation: the addition of nutrients or growth enhancing co-substrates and/or improvement in habitat quality to stimulate growth of indigenous bacteria (Ogbonna *et al*., 2007)

Phytoremediation is a relatively efficient, environmentally-friendly and promising technology for removing many contaminants such as hydrocarbon pollutants. The synergy between plant roots and soil microorganisms promotes the degradation of persistent organic contaminants in phytoremediation. Removal of petroleum hydrocarbons from soil in phytoremediation is often attributed to microorganism living in the rhizosphere, under the influence of plant roots (Luepromchai *et al*., 2007). Microbial communities in planted soils are greater and more active than in unplanted soils (Johnson *et al*., 2005; Mueller and Shann, 2006). Microorganisms that live in the rhizosphere benefit from the root exudates and plants in return benefit from the metabolic detoxification of potentially toxic compounds brought about by microbial communities. Additionally, plants benefit from the presence of microbial populations through the recycling and solubilisation of mineral nutrients that stimulate plant growth (Escalante-Espinosa *et al*., 2005).The degradability of crude oil hydrocarbon (alkanes, alkenes, aromatics and polars) in soil, sludge, sediments, and marine environment by naturally-occurring microbes have been documented (Salanitro *et al*., 1997).

Experiments have shown that differences in the extent of soil hydrocarbon biodegradation may depend upon soil and crude oil types, concentration of total applied hydrocarbon, and nutrient growth stimulants (e.g NH_3 and PO^{3-}_4) based on optimum C:N:P ratios (Salanitro *et al*., 1997). Other studies showed that light-medium (API gravity 39 and high saturate fraction) crude oil biodegraded (O_2 uptake and reduction in oil and grease) more extensively than a heavier crude (API gravity 21). Few studies have identified the fraction and types of petroleum hydrocarbons that are readily degraded or recalcitrant in oily waste soil treatment systems. Huesemann and Moore (1993) showed that 93% of the saturate and 79% of the aromatic compounds having carbon numbers in the range of C_{10}-C_{44} were degraded in a sandy soil containing weathered Michigan (medium API gravity) crude oil with an initial concentration of 30,000 mg/kg TPH. In this same study, however, the polar fraction was resistant to microbial metabolism and did not degrade during the study 5.5-month test. In another report by Heusemann (1995) on the limits and extent of bioremediating TPH in different oily soils showed that 90% of the alkanes and monocyclic saturates and 50-70% of aromatic compounds (< C_{44}) were degraded. The study showed that overall bioremediation effectiveness was dependent upon hydrocarbon types present and was not affected as much by soil type, nutrient fertilizer addition, microbial populations, or treatment conditions (slurry versus static soil conditions). It showed also that saturate and aromatic compounds with polycyclic structures were most resistant to removal by enhanced soil biotreatment methods.

The apparent recalcitrance of petroleum hydrocarbon fractions may be due to factors such as lack of bioavailability (inaccessible because of soil sorption and uptake by soil microbes), lack of requisite oxidizing enzymes, and/or steric hindrance for enzyme attack and toxicity to soil microorganisms (Salanitro *et al*., 1997). Salanitro *et al.* (1997) studied the bioremediation of three crude oils (heavy, medium and light of API gravity 14, 30, and 55, respectively) with 4000-27000mg/kg TPH. The results obtained followed a first-order removal rates in which 50-75% and 10-90% of the total petroleum hydrocarbons (TPH) were degraded in 3-4 months for low and high organic soils respectively. The gas chromatographic profiles showed that, after bioremediation, hydrocarbons in oily soils decreased by 70-90%, 40-60%, and 35-60% for those carbon number species in the range of C_{11}-C_{22}, C_{23}-C_{32} and C_{35}-C_{44}, respectively. Bioremediated soils were neither toxic to earthworms, inhibitory in the Microtox test, nor inhibited seed germination after 5 (high organic soil) or 10-12 (low organic soil) months of treatment.

Wood ash is a significant waste by-product from the burning of wood as fuel that has beneficial reuse as forest fertilizer, as practiced in Scandinavia (Pitman, 2006). Ash is successfully used elsewhere on agricultural land, for other purposes such as sewage amendment, scrubber systems, cement products (Greene, 1988) and for road building in both Scandinavia and the USA (Pitman, 2006). Wood ash has been used as soil ameliorant for second-rotation conifer stands on drained peats, restoration of cut-over peat using ash as an ameliorant to change pH and restore biodiversity, and restoration of acidified soil. Elemental composition varies with the type of plant tissue included in the wood fuel. The composition of wood ash is also dependent on the tree species burnt. Wood ash contains Ca, Fe, K, Mg, Mn, Na, P, S, Al and C; but N is rarely reported (Pitman, 2006).

Polyaromatic hydrocarbons (PAHs) occurrence in wood ash are generally the less toxic compounds with naphthalene the most common (Diebel *et al*., 1992). Ash is essentially hydrophilic and absorbs water into pores by capillary action simultaneously with chemical changes of hydration of oxides. Vance (1996) suggested that one single application of wood ash could replace nutrient losses from whole-tree harvesting sites, along with additional N amendments to create balance input. The dramatic effect of wood ash application on tree growth was reported by Ferm *et al*. (1992) who affirmed a standing volume increase from 15 to 70m^3ha^{-1} at the highest rate of application. They also reported peat decomposition, indicating enhanced N mineralization under the ash. Seedling root extensions are encouraged by ash additions and have shown greater fine root length extension in wood ash-treated fertilization (Pitman, 2006).

In this study, it was thought that one of the major soil condition created by the presence of crude oil in soil is hydrophobicity or inability of the contaminated soil to absorb water for plant growth. The objectives of this study were:

1) To use ash from wood as supplement in crude of polluted soil with very high TPH
2) Growth of plant in crude oil polluted soil supplemented with wood ash
3) Determine the compositional changes following degradation of crude oil polluted soil after plant growth

MATERIALS AND METHODS

Collecting of Soil Sample

Crude oil polluted soil and unpolluted soil samples were collected from an oil-field location owned by Nigerian Petroleum Development Company (NPDC), Benin City. The soil samples collected weighted over 50kg each.

Collection of Plant Species

Seeds of *Corchorus aestuans* L. were obtained from NIHORT while seeds (calabash) of *Lagenaria siceraria* (Mol.) Standl. were collected from local farmers.

Collection of Wood Ash

Over 5kg of wood ash were collected from one of the kitchen of central bukateria complex in the University of Benin, Benin City, Nigeria.

Soil Preparation for Field Study

The soil samples were prepared into the following polluted categories-0%, 50% and 100% crude oil polluted soil samples. The crude oil polluted soil obtained from the flow station was divided into two parts`. The first part was used to prepare 50% polluted soil samples by weighing 1kg of polluted soil and mixing it with another 1kg of unpolluted soil. Mixing was done thoroughly for evenness of soil matrix before packaging into polypots. From the other part, 2kg polluted soils were put into polypots to make 100% polluted soil samples. Similarly, the 0% soil samples were prepared by weighing 2kg of unpolluted soil obtained from the oil field location in poly pots.

Soil Treatment with Wood Ash

In this study, weights of ash supplements used were 0g, 10g and 20g in unpolluted or crude oil polluted soil samples. These weights of ash were thoroughly mixed with the selected soil sample in the polypots to obtain the following treatments.

Table 1: Soil treatments and ash supplementation used in the study

Soil Sample	Weight or ash applied	No of poly pots
Unpolluted soil	No ash applied	5
	10g ash applied	5
	20g ash applied	5
50% polluted soil	No ash applied	5 (2)
	10g ash applied	5 (2)
	20g ash applied	5 (2)
100% polluted soil	No ash applied	5 (2)
	10g ash applied	5 (2)
	20g ash applied	5 (2)

Figures in parentheses indicate additional poly pots prepared for the purpose of observing degradation of polluted soil without plant growth

When the treatment with ash was completed, the pots were transferred to the field, watered and left in the field overnight before sowing of seeds.

Experimental Design

This stud was conducted as a completely randomized designed with five replicates. Mean and standard deviation were calculated.

Planting of Seeds and Field Data Measurement

In this study, two species of plants were grown successively, one after the other. The first species that was sown into the soil samples was *Corchorus aestuans*. Thirty seeds were sown into each poly pot. Forty days after planting (40 DAP), more than 95% of the plants in both 50% and 100% polluted soil samples withered. This necessitated the sowing of *Lagenaria siceraria* seeds in the poly pots. Five seeds of *Lagenaria siceraria* were sown per pot. Germination was recorded on the appearance of the cotyledons above the soil surface. This record was take for 14 days.

$$\%\ germination = \frac{No\ of\ seeds\ that\ germinated}{No\ of\ seeds\ sown}\ x\ 100$$

Plant height measurements were taken after 2 weeks of growth in the field, i.e 14 DAP. This was carried out using a metre rule or flexible measuring tap. Stem circumference was taken once every two weeks. This was carried out using a threat to encircle the stem at the first internode and determine its length on a metre rule. Leaf elongation rate was recorded using the third and fourth leaves from the apex. The lengths of the leaves were measured from the base of the stalk to the leaf tip. These measurements were carried out consecutively for 5 days when the plant were 2 weeks old and repeated when the plants were 4 weeks old. Calculation was as follows:

$$Leaf\ elongation\ rate = \frac{(L_5 - L_4) + (L_4 - L_3) + (L_3 - L_2) + (L_2 - L_1)}{4}$$

L_1, L_2, L_3, L_4 and L_5 refer to measurement of leaf length (including stalk) for day 1, day 2, day 3, day 4 and day 5 respectively.

Total Petroleum Hydrocarbon Determination of Soil Sample obtained from Field

Five grammes (5g) of the wet-soil sample was put in 50ml glass bottle containing about 5g anhydrous sodium sulphate. Large particles of gravel or stones were excluded from the soil sample because they are capable for resulting in highly variable data. The soil sample-anhydrous sodium sulphate mixture was stirred until it became free flowing mixture. Anhydrous sodium sulphate was added more when the mixture was observed to be wet still. This mixing was done quickly to limit loss of volatile fraction. Add 20ml of dichloromethane was added and shaken vigorously for 5 minutes using a vortex mixer. After shaking, the mixture was allowed to stand for 15 minutes and supernatant obtained by filtering. Pour 1ml of soil extract into 15ml-screw capped vial containing 5ml dichloromethane. Add 0.1g aluminium oxide, seal and shake for about one minute. Add about 0.1g aluminium chloride, seal and shake intermittently for 10 minutes. Allow the vial to stand for 15-20 minutes, leaving a clear coloured solvent extract and read absorbance at 420nm against a blank made up of 6ml dichloromethane plus 0.1g aluminium oxide and 0.1g aluminium chloride (but no soil). The total petroleum hydrocarbon is calculated:

$$TPH\ (mg/kg\ soil) = \frac{Absorbance\ x\ DF\ x\ 20}{Weight\ of\ soil\ used\ (g)}$$

Where DF = Dilution factor

20 = Initial extraction volume (ml)

pH was air-dried for 3 days before pH was determined. Determination was done in a soil-water slurry (1:1) and read pH value using a pH meter.

GC-FID Soil Analysis

a) **Soil Sample Collected:** After the termination of the experiment, the plants were harvested from the soil. The soils in the experimental pots were mixed in order to obtain a homogenous mixture before soil samples were collected using stainless steel scoop. Soil samples collected this way were sent to the laboratory for GC-FID analysis. Samples collected were designated as:
E1 – Unpolluted soil + 10g ash with plant
E2 – Unpolluted soil + 20g ash with plant
E3 – 50% polluted soil + 10g ash, no plant
E4 – 50% polluted soil + 10g ash with plant
E5 – 50% polluted soil + 20g ash, no plant
E6 – 50% polluted soil + 20g ash with plant
E7 – 100% polluted soil + 10g ash, no plant
E8 – 100% polluted soil + 10g ash with plant
E9 – 100% polluted soil + 20g ash, no plant
E10 – 100% polluted soil + 20g ash with plant
E11 – 100% polluted soil (stored at field environmental conditions)
P_0 – Uncontaminated soil obtained from field
P_{100} – Polluted soil obtained from field

b) Soil Sample Extraction

A solvent mix of acetone and methylene chloride (50:50) was prepared. Ten grammes (10g) of soil samples was put in a beaker and 50ml of solvent mix was added. Spike with 1ml of the surrogate mix. The vessels were tightly capped and thoroughly mixed for 5 minutes, sonicated for 30 minutes at 70^0C. Add up to 10g of anhydrous sodium sulphate to the sample until a clear extract develops. Pour the extracted solvent into a round bottom flask. Repeat once more by adding 50ml of solvent mix, sonicate, allow to stand for 20 minutes and decant into another flask. Concentrate the extracted solvent until a residue was obtained using an evaporator. To this residue, add 10ml of hexane and re-concentrate to 1-3ml. The sample is ready to be fractionated into the aliphatic and aromatic fractions using silica gel columns. Pack the columns with 10g of 100-200 mesh silica gel pre-conditioned (baked) at 105^0C overnight. Mix the silicate with hexane to form slurry.

c) **GC analysis for aliphatic and aromatic hydrocarbons:**

Analysis were performed with a gas chromatograph (system 6890 series) equipped with HP-5 capillary column (30m length x 0.25mm internal diameter x 1.00µm film thickness) and a flame ionization detector (FID) (Hewlett-Packard Co., Palo Alto, CA, USA) and splitless injector. All runs were conducted under the following conditions: initial temperature was 60^0C, isothermal for 1 minute, temperature rate was 10^0C per minutes, final temperature was 310^0C, isothermal for 5 minutes. The injector was at 250^0C, FID at 320^0C and helium was the carrier gas. For the aliphatic hydrocarbon set up, temperature programme was initial temperature 60^0C, initial time 2 minutes, rate 8^0C/minutes, final temperature 300^0C, final temperature 300^0C, detector temperature 300^0C, aromatic hydrocarbon setup; temperature programme was initial temperature 100^0C, initial time 1 minute, rate 4^0C/minute, final temperature 310^0C, detector temperature 300^0C. Injected sample volume was 1µL. Before extracts were analysed, the instrument was calibrated by injecting series of normal alkane standards. Response factors for each alkane calculated using the area response and the amount of standard material. This was used for the identification of crude oil constituents.

RESULTS

The results obtained in this study are shown in Tables 2-3 and Figures 1-30. The total petroleum hydrocarbon (TPH) content analyses of the soil samples obtained from the field were 10.90 ppm and 40,173.90 ppm for uncontaminated and polluted soils respectively. The pH values obtained were 6.26 and 4.67 for uncontaminated and polluted soils respectively. The high TPH of polluted soil was envisaged because at the point of collection, it was observed that the environment may have been recently polluted and odour or smell of fresh crude oil was strong. The seed germination of *Corchorus aestuans* was observed in all soil treatment six days after sowing.

The germination results showed that for oil polluted soil samples, the percentage germination were 40% and below (see Table 2) fourteen days after planting. The germination of *Lagenaria siceraria* seeds is shown in Table 3. Germination of seeds of *Lagenaria siceraria* in oil polluted soil samples supplemented with 10g or 20g wood ash were higher than what were obtained for *Corchorus aestuans.* Plant height measurements showed that oil contamination decrease values obtained comparing unpolluted and polluted (Figures 1 & 2). In *Corchorus aestuans,* 20g wood ash supplementation of 50% and 100% polluted soil produced higher values than 10g ash after 4 weeks of growth (Figure 1). Promotion of vegetative growth by ash supplementation was observed in plants grown on uncontaminated soil (Figure 2). Other vegetative data recorded are shown in Figures 3 and 4. Aromatic and aliphatic hydrocarbon contents were detected in unpolluted and polluted soils (Figures 5-8) obtained from the field before planting. The peaks were easily recognizable in the chromatograms of the polluted soil. Other chromatograms showed detection in soil samples either after plant growth or without plant five months after initiation of study (Figures 9-30). Generally, the reduction in hydrocarbon contents was apparent following plant growth. Ash supplementation of polluted soil facilitated the degradation of crude oil in soil. For example, 100% polluted soil samples, the TPH (mg/Kg) obtained for soil without ash supplementation was 1.292, soil with 10g ash supplement with no plant was 1.027 and soil with 20g ash supplementation with no plant was 1.221, at the termination of the study. Where plants were grown, the TPH values (mg/Kg) were 0.580 and 0.158 for 10g ash plus plant and 20g ash plus plant respectively. This suggests that 20g ash supplementation enhanced crude oil degradation in soil more than when no ash or 10g ash were involved. Also the plant growth contributed to the crude oil degradation as well.

Table 2: Germination of *Corchorus aestuans* L. in unpolluted and polluted soils supplemented with wood ash

	DAYS AFTER SOWING				
Soil conditions	**3**	**6**	**9**	**12**	**14**
0%, no ash	-	25.63±8.08	43.34±20.82	56.65±23.29	59.00±25.36
0%, 10g ash	-	25.64±15.04	37.67±15.01	54.68±23.54	57.56±25.01
0%, 20g ash	-	14.65±4.16	34.31±6.51	51.66±4.73	62.00±5.00
50%, 10g ash	-	9.25±2.87	13.25±2.85	13.25±2.85	32.50±5.37
50%, 20g ash	-	10.00±2.45	30.75±14.91	36.75±6.95	40.00±7.26
100%,10g ash	-	10.75±2.85	16.75±9.75	21.75±15.88	31.75±15.22
100%, 20g ash	-	20.25±8.30	30.00±16.51	32.50±13.58	32.50±13.58

Figures = mean ± S.D.

Table 3: Germination of Lagenaria siceraria (Mol.) Standl. in unpolluted and polluted soils supplemented with wood ash

	DAYS AFTER SOWING				
Soil conditions	**3**	**6**	**9**	**12**	**14**
0%, no ash	-	30.00±20.45	35.75±10.10	42.85±20.15	42.85±20.15
0%, 10g ash	-	25.00±15.00	30.50±5.00	35.00±9.50	35.00±9.50
0%, 20g ash	-	52.37±21.79	57.30±14.25	57.30±14.25	57.30±14.25
50%, 10g ash	-	57.13±14.25	60.70±17.96	67.85±21.40	67.85±21.40
50%, 20g ash	-	35.75±8.23	60.70±13.60	60.70±13.60	64.00±18.10
100%,10g ash	-	10.00±8.00	19.00±8.31	23.75±16.55	28.50±24.77
100%, 20g ash	-	28.55±14.35	47.63±8.79	47.63±8.79	47.63±8.79

Figures = mean ± S.D.

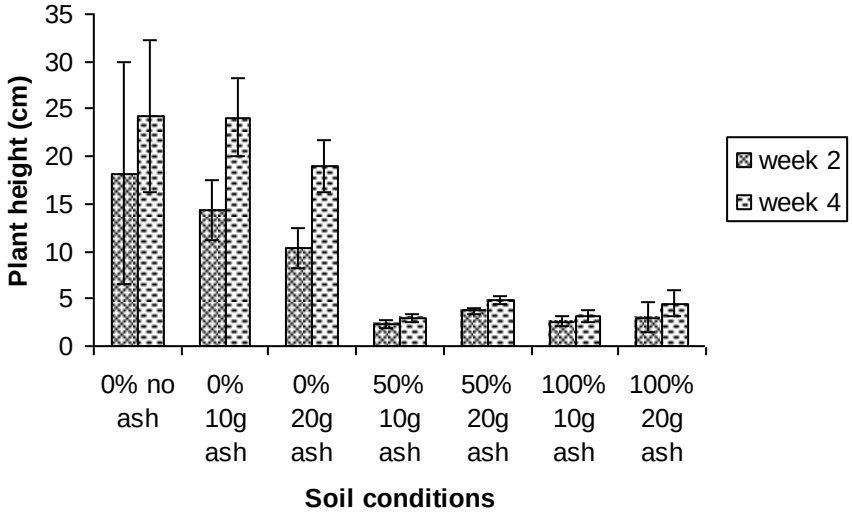

Figure 1: Plant height of *Corchorus aestuans* in unpolluted and polluted soils supplemented with wood ash

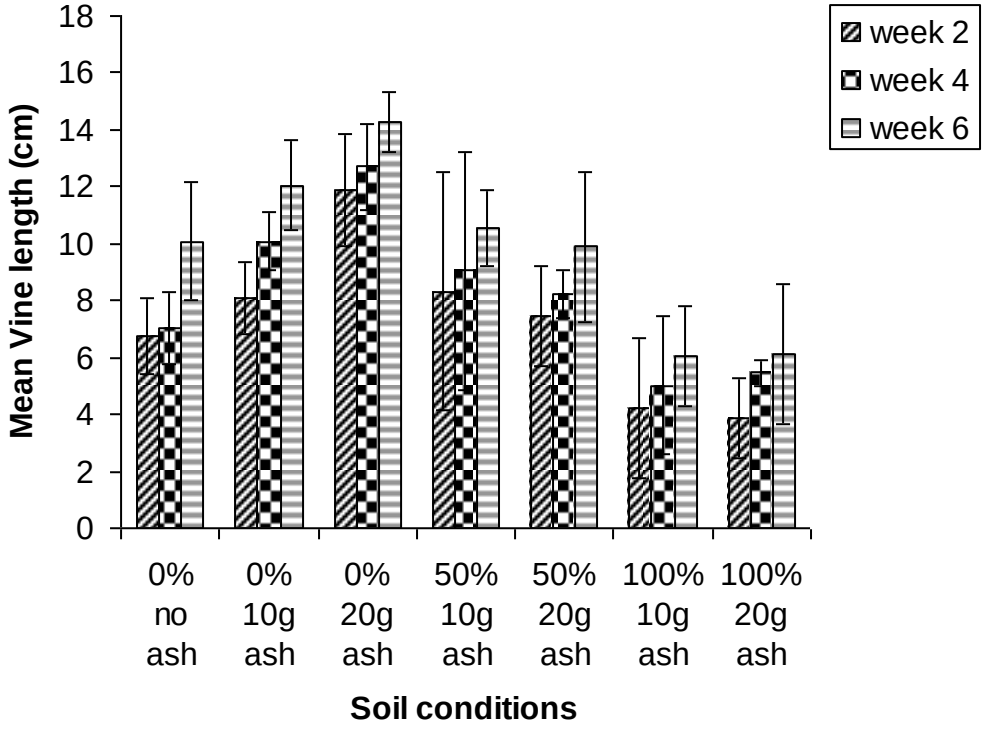

Figure 2: Vine length of Lagenaria siceraria in unpolluted and polluted soils supplemented with wood ash

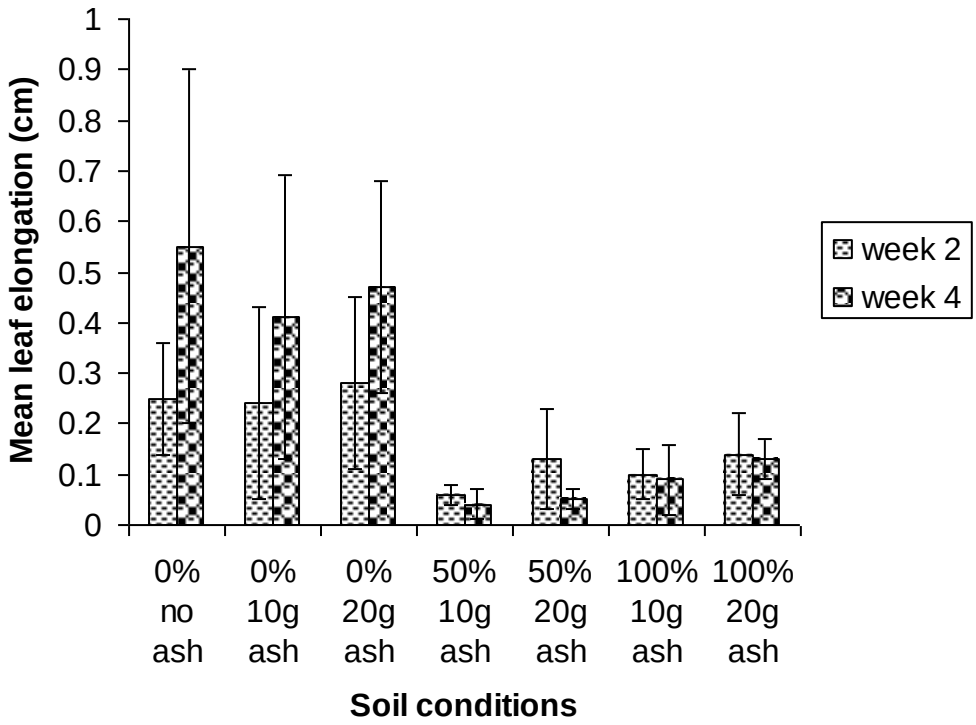

Figure 3: Leaf elongation of *Lagenaria siceraria* in unpolluted and polluted soils supplemented with wood ash

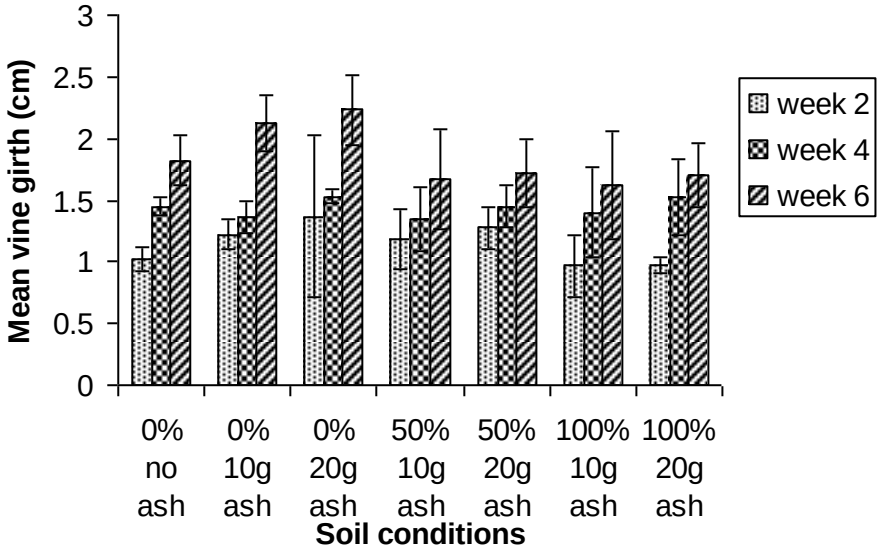

Figure 4: Vine girth of *Lagenaria siceraria* in unpolluted and polluted soils supplemented with wood ash

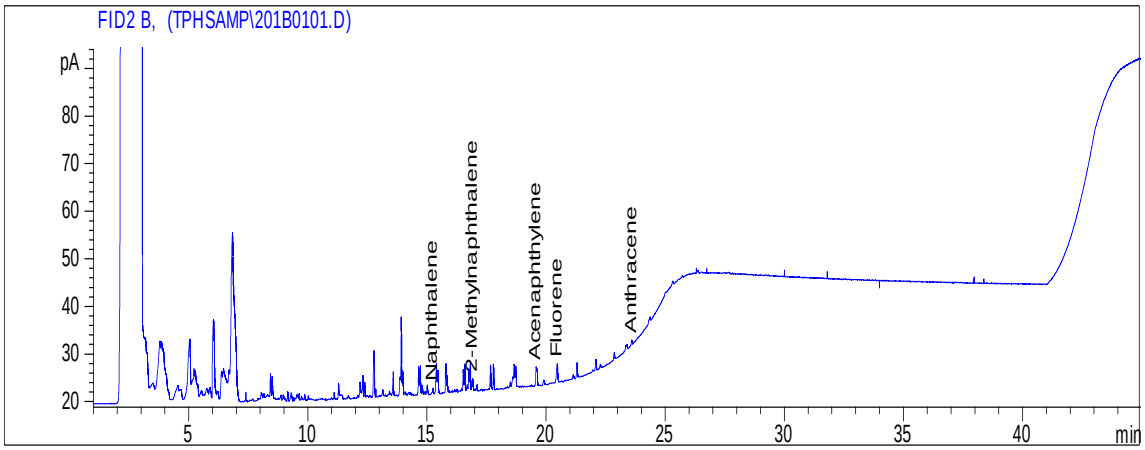

Figure 5: Chromatogram of aromatic hydrocarbon content in unpolluted soil obtained from the field

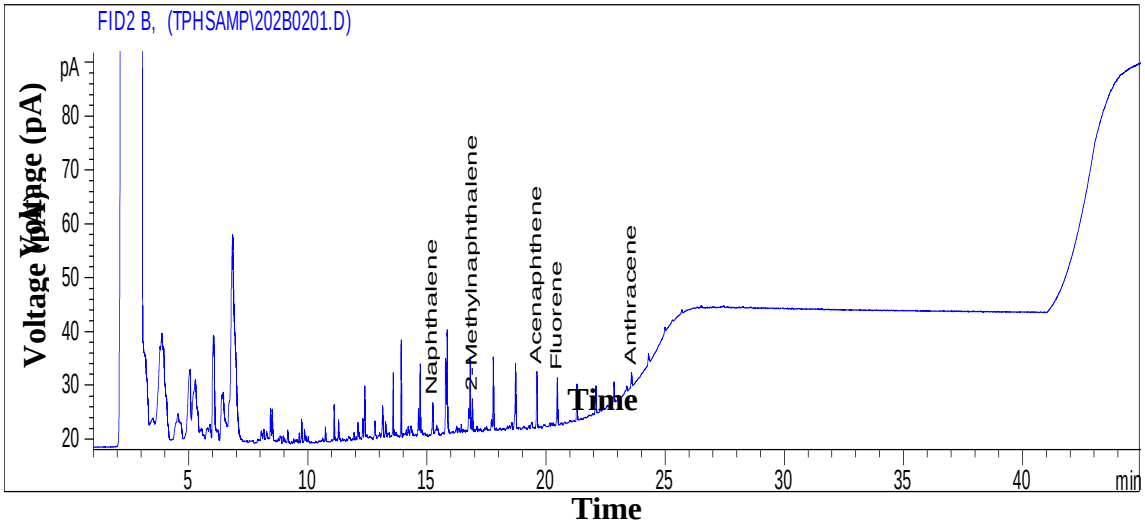

Figure 6: Chromatogram of aromatic hydrocarbon content in crude oil polluted soil obtained from the field

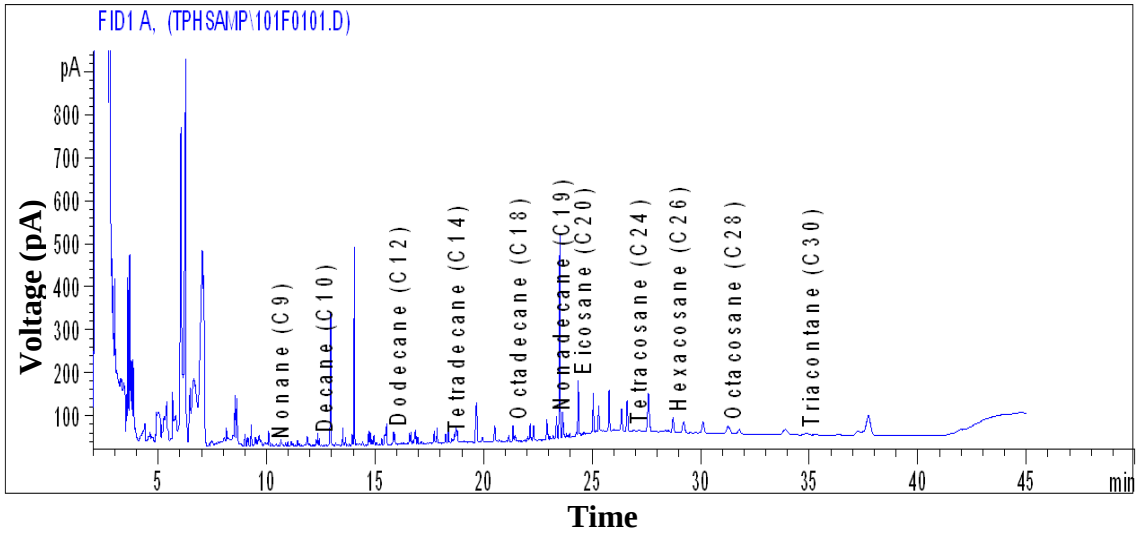

Figure 7: Chromatogram of aliphatic hydrocarbon content in unpolluted soil obtained from the field

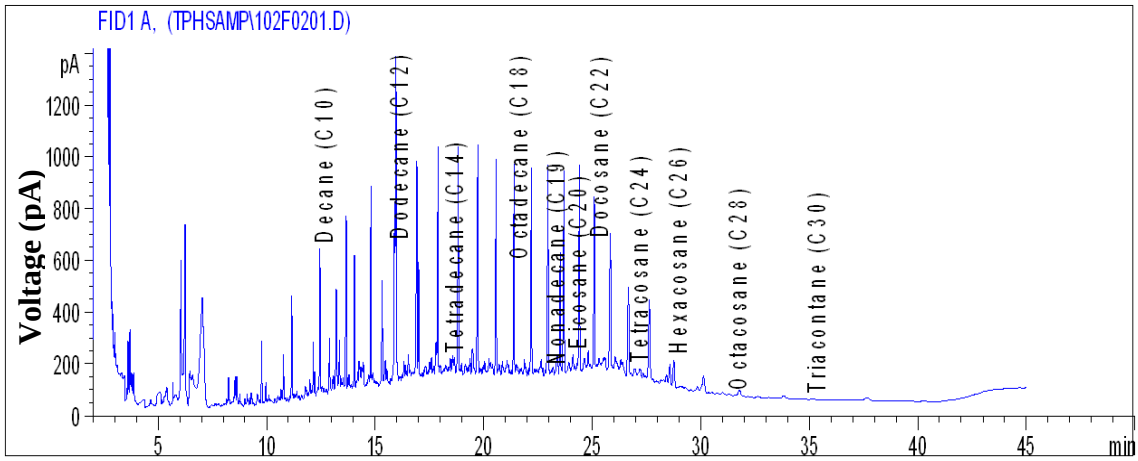

Figure 8: Chromatogram of aliphatic hydrocarbon content in crude oil polluted soil obtained from the field

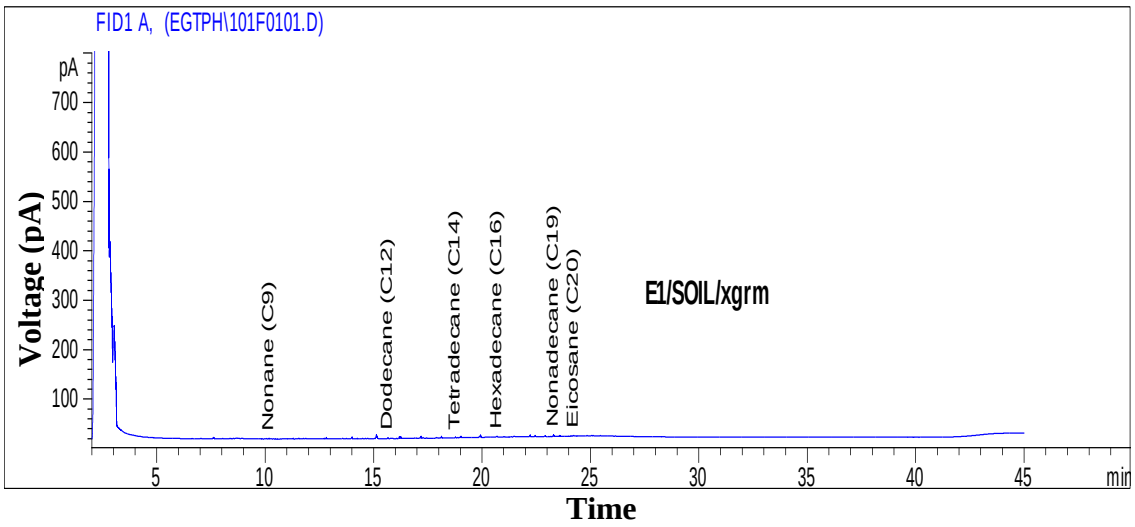

Figure 9: Chromatogram of aliphatic hydrocarbon content in unpolluted soil supplemented with 10g ash after plant harvest

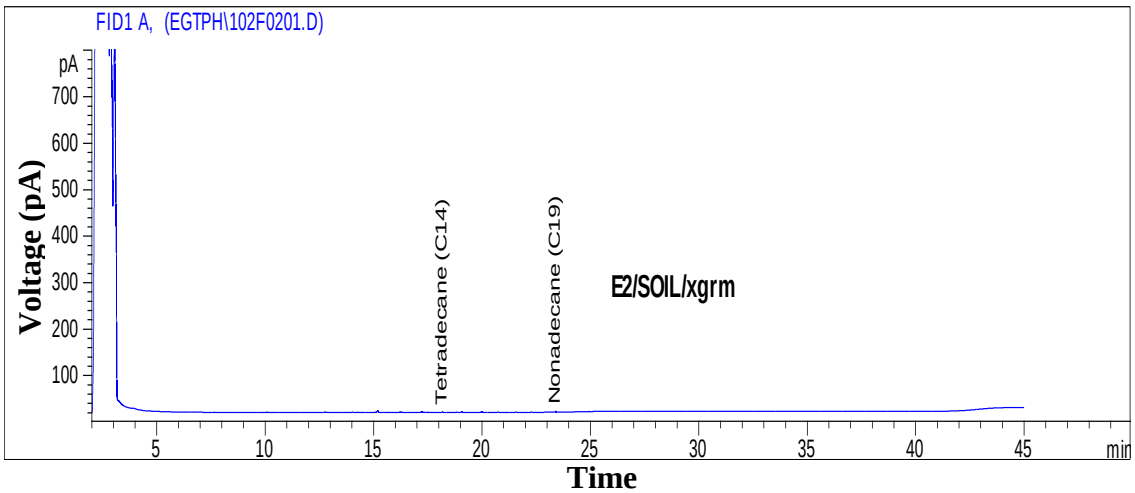

Figure 10: Chromatogram of aliphatic hydrocarbon content in unpolluted soil supplemented with 20g ash after plant harvest

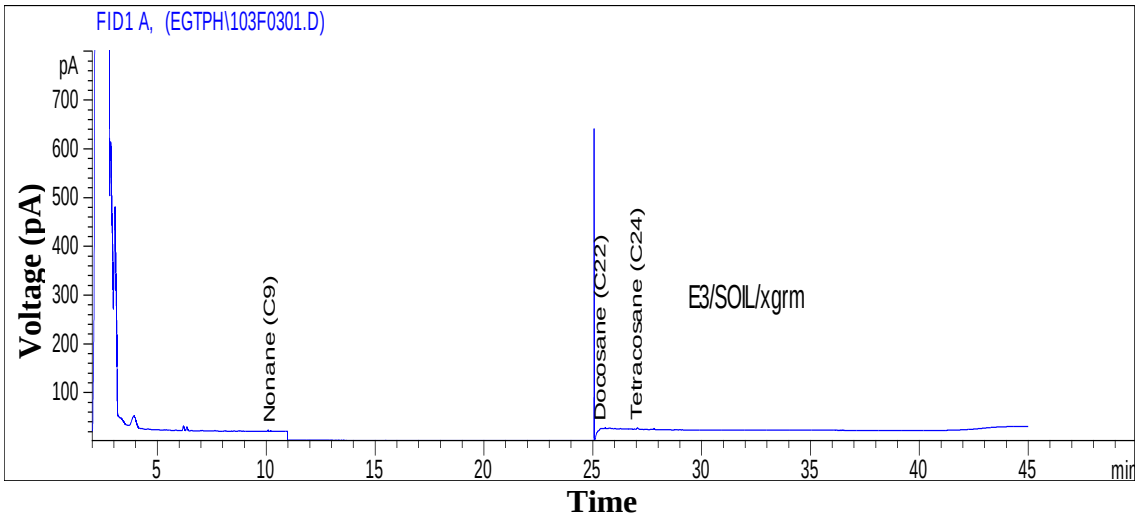

Figure 11: Chromatogram of aliphatic hydrocarbon content in 50% polluted soil supplemented with 10g ash without plant growth

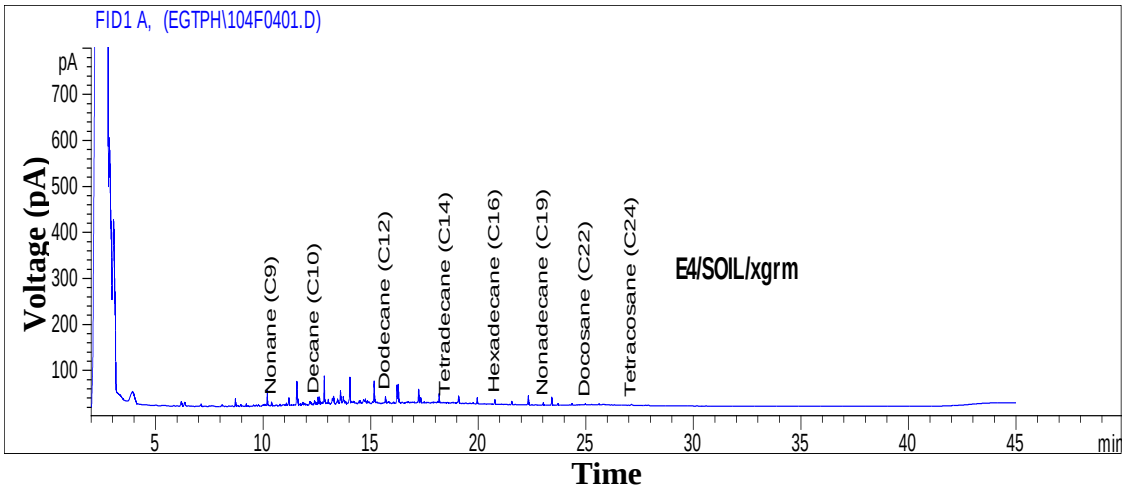

Figure 12: Chromatogram of aliphatic hydrocarbon content in 50% polluted soil supplemented with 10g ash after plant harvest

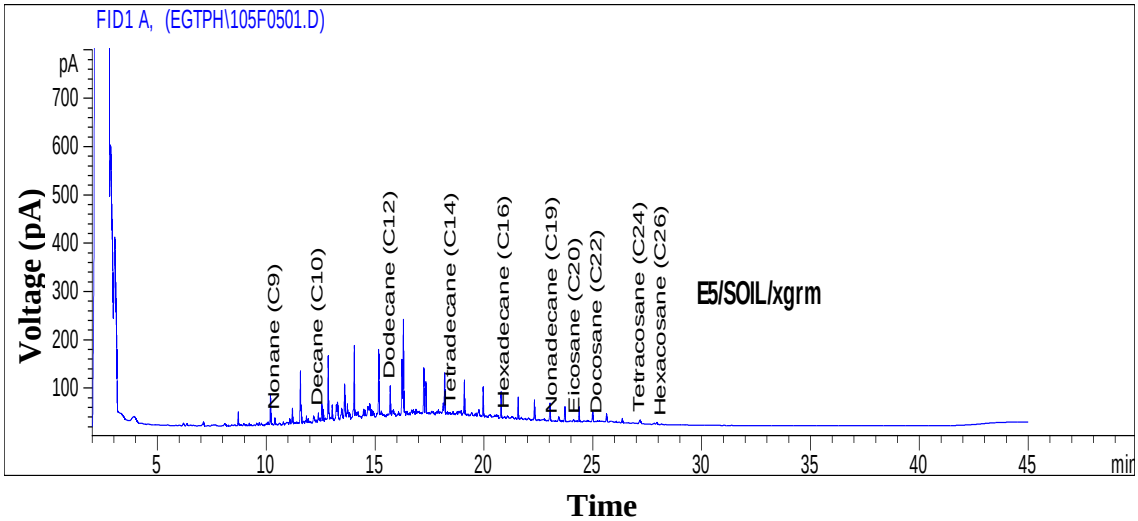

Figure 13: Chromatogram of aliphatic hydrocarbon content in 50% polluted soil supplemented with 20g ash without plant growth

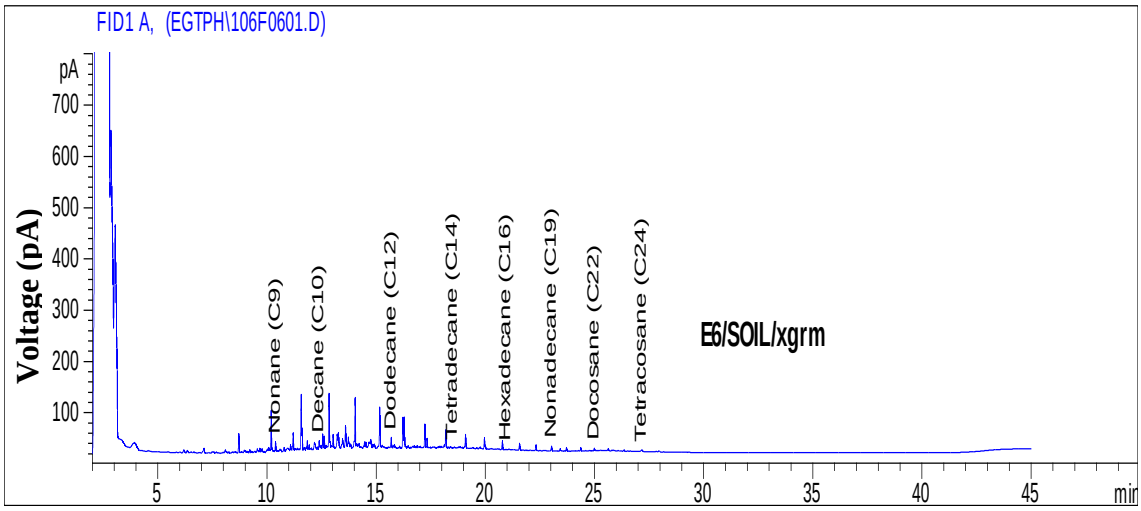

Figure 14: Chromatogram of aliphatic hydrocarbon content in 50% polluted soil supplemented with 20g ash after plant harvest

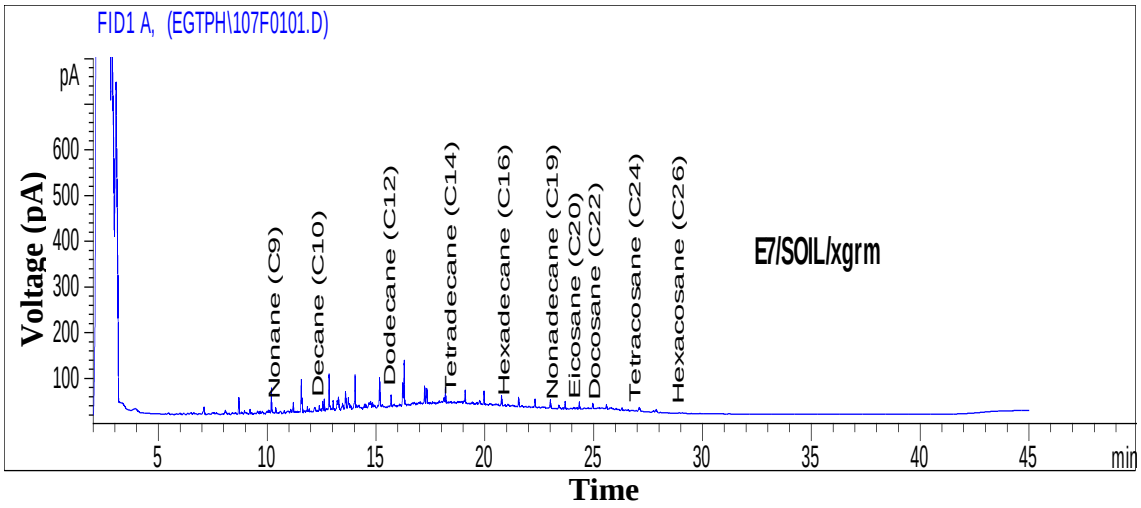

Figure 15: Chromatogram of aliphatic hydrocarbon content in 100% polluted soil supplemented with 10g ash without plant

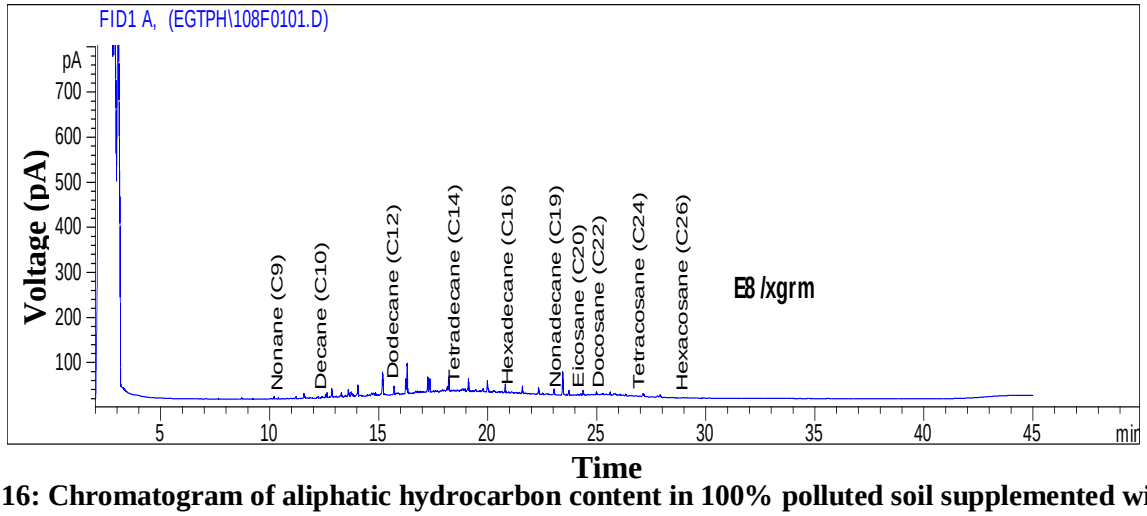

Figure 16: Chromatogram of aliphatic hydrocarbon content in 100% polluted soil supplemented with 10g ash after plant harvest

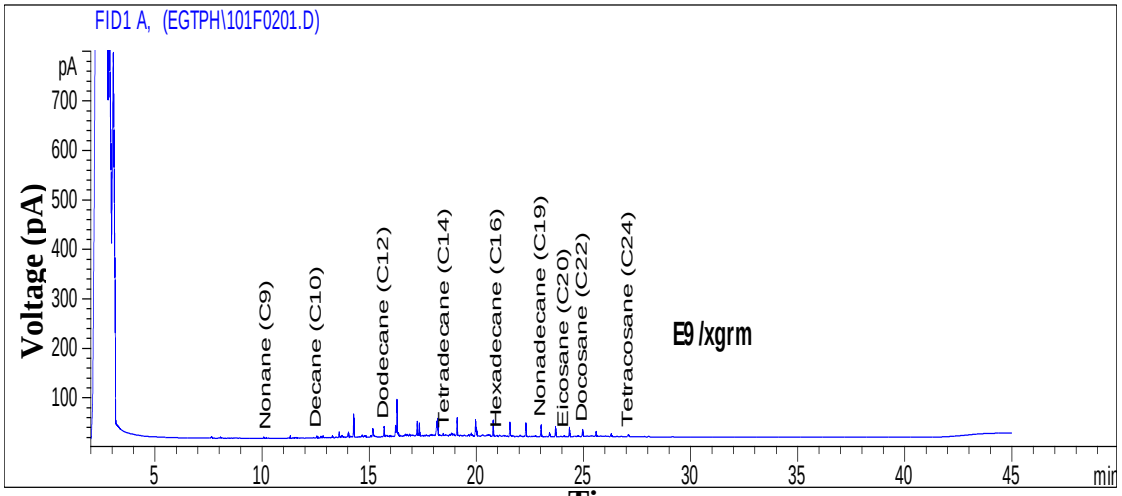

Figure 17: Chromatogram of aliphatic hydrocarbon content in 100% polluted soil supplemented with 20g ash without plant

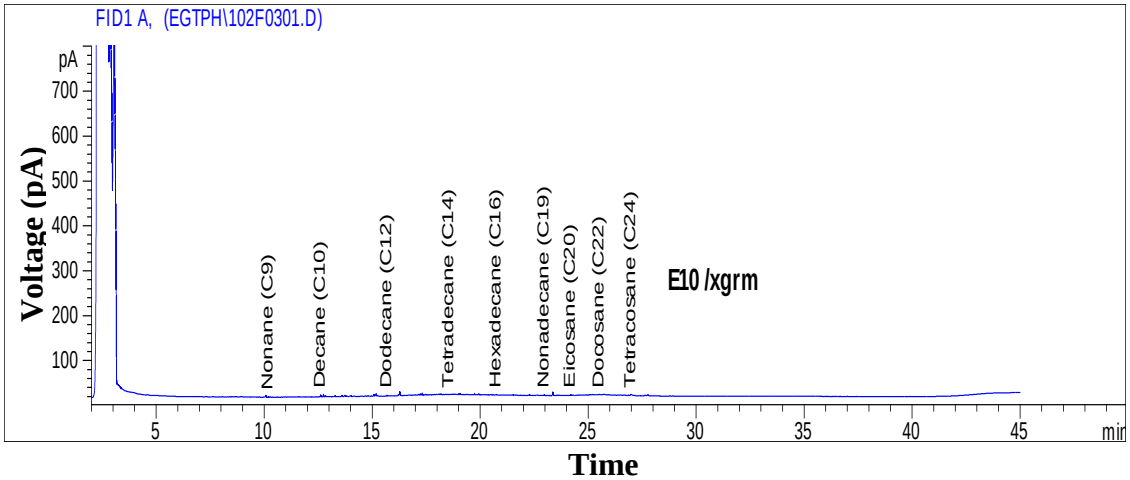

Figure 18: Chromatogram of aliphatic hydrocarbon content in 100% polluted soil supplemented with 20g ash after plant harvest

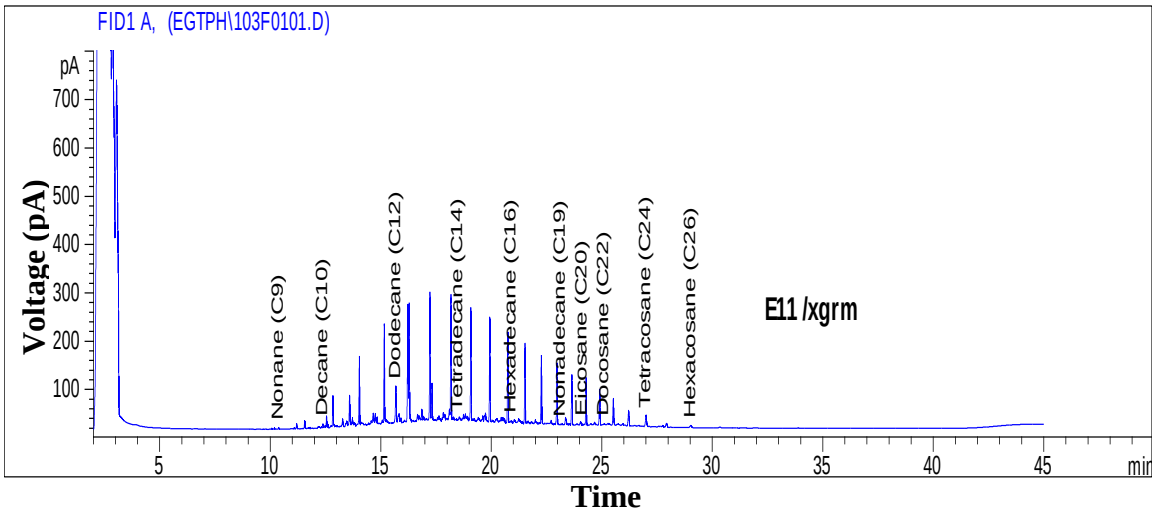

Figure 19: Chromatogram of aliphatic hydrocarbon content in 100% polluted soil without ash and plant left under field condition for the duration of the study.

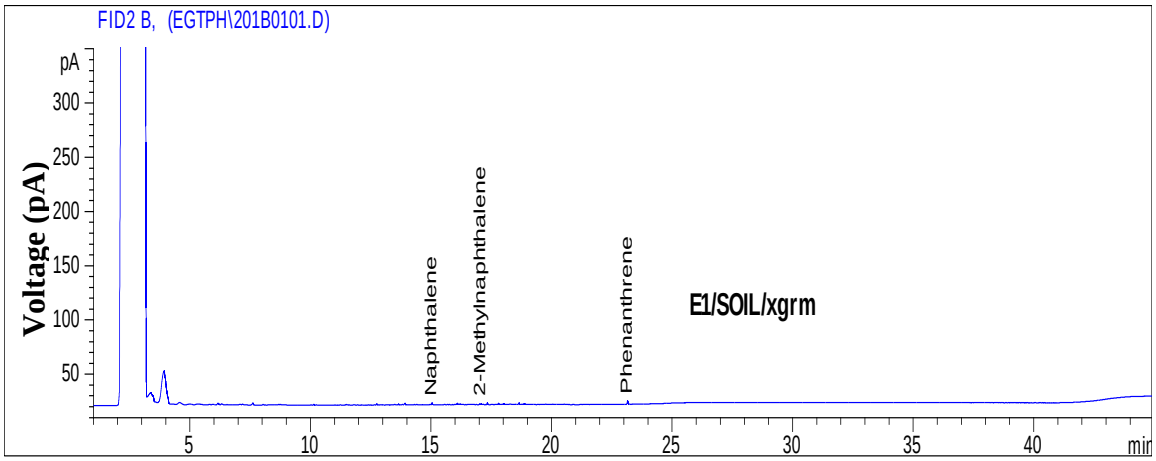

Figure 20: Chromatogram of aromatic hydrocarbon content in unpolluted soil supplemented with 10g ash after plant harvest

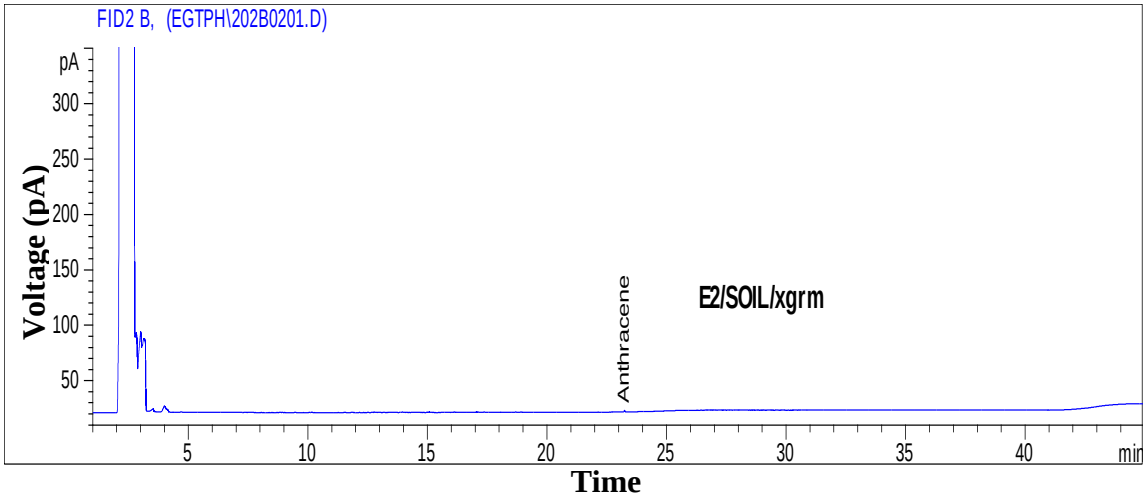

Figure 21: Chromatogram of aromatic hydrocarbon content in unpolluted soil supplemented with 20g ash after plant harvest

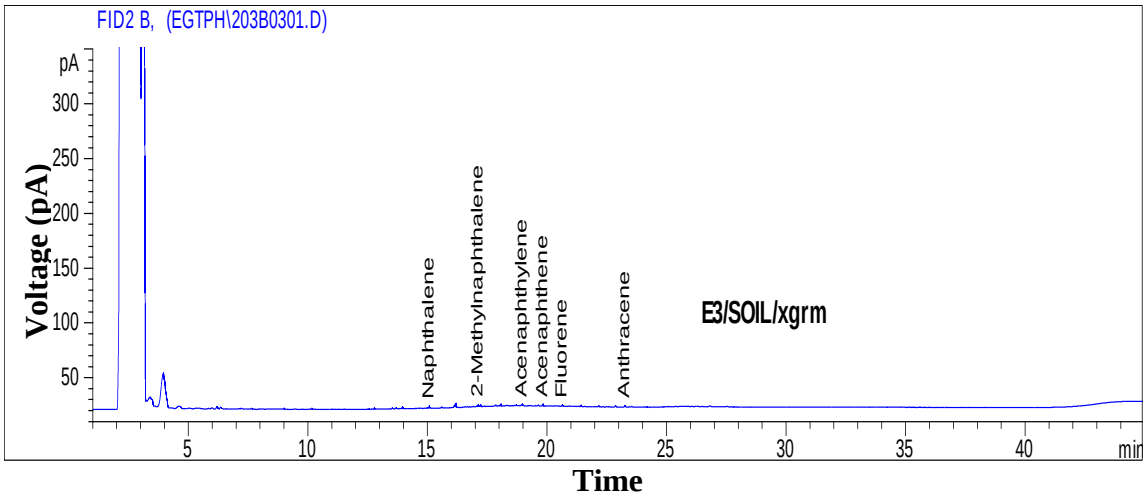

Figure 22: Chromatogram of aromatic hydrocarbon content in 50% polluted soil supplemented with 10g ash without plant

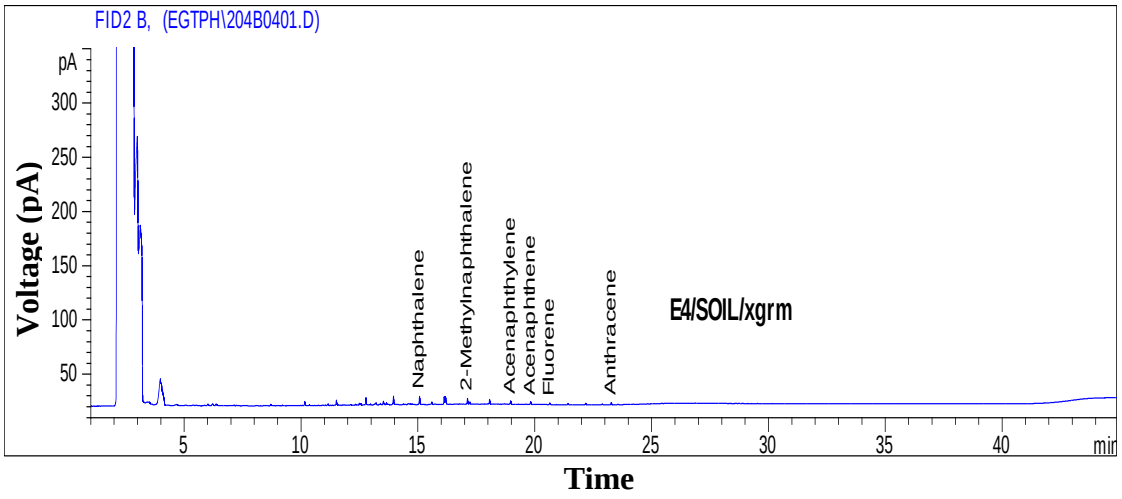

Figure 23: Chromatogram of aromatic hydrocarbon content in 50% polluted soil supplemented with 10g ash after plant harvest

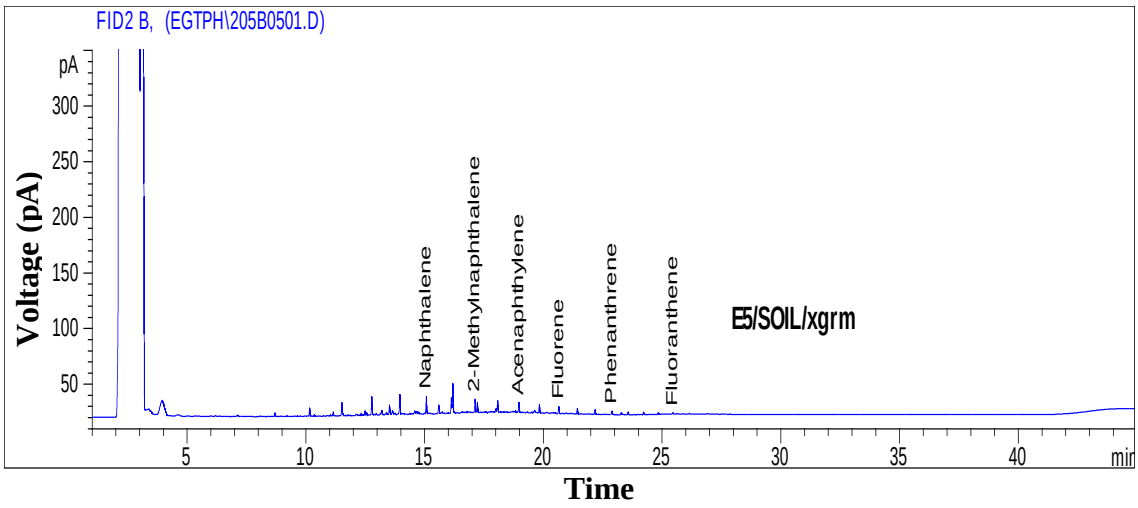

Figure 24: Chromatogram of aromatic hydrocarbon content in 50% polluted soil supplemented with 20g ash without plant

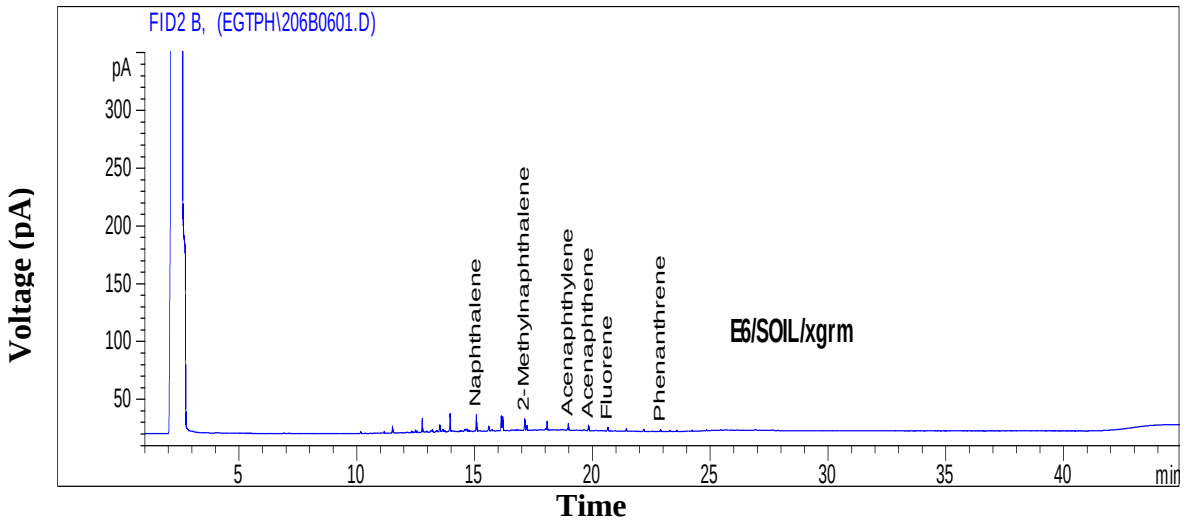

Figure 25: Chromatogram of aromatic hydrocarbon content in 50% polluted soil supplemented with 20g ash after plant harvest

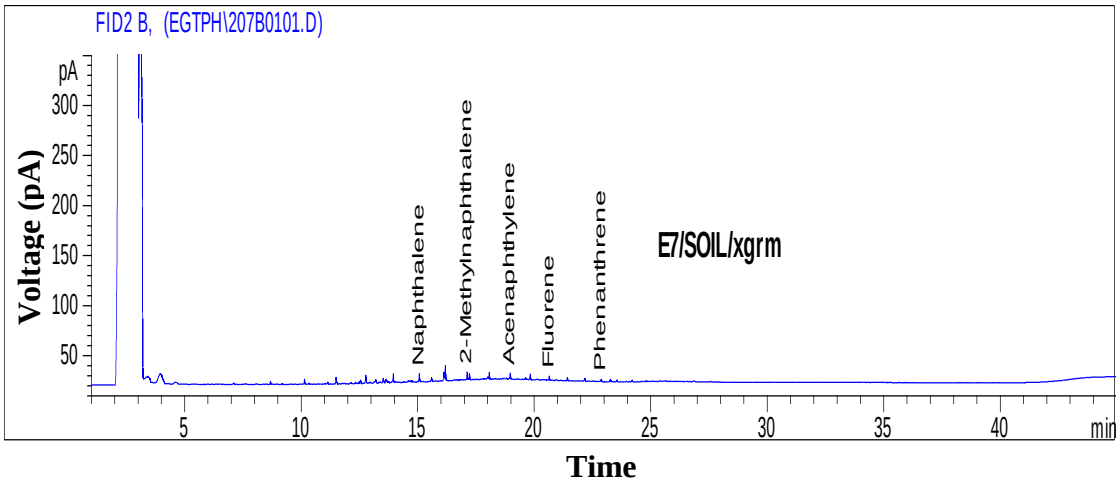

Figure 26: Chromatogram of aromatic hydrocarbon content in 100% polluted soil supplemented with 10g ash without plant

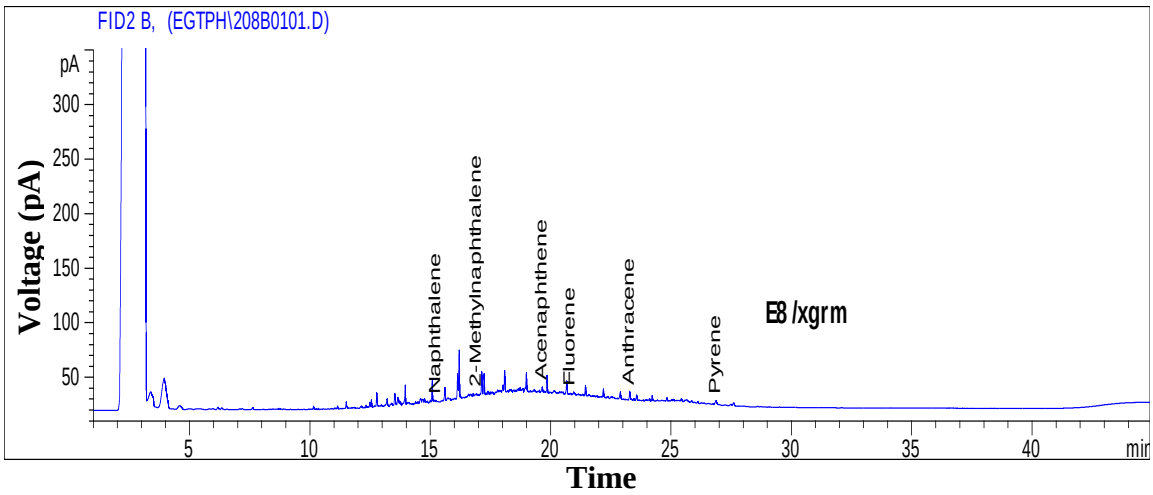

Figure 27: Chromatogram of aromatic hydrocarbon content in 100% polluted soil supplemented with 10g ash after plant harvest

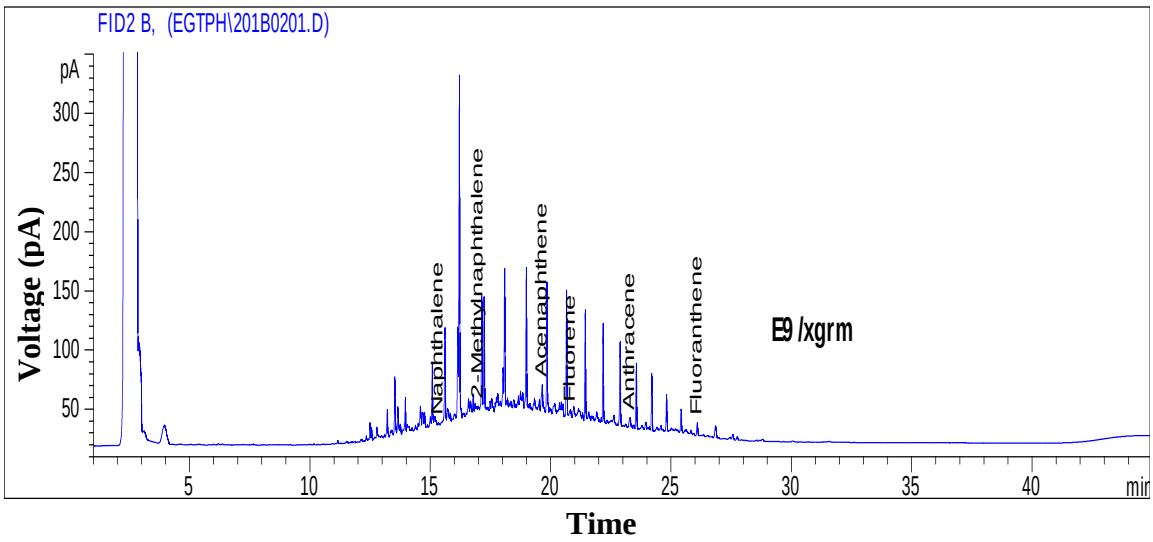

Figure 28: Chromatogram of aromatic hydrocarbon content in 100% polluted soil supplemented with 20g ash without plant

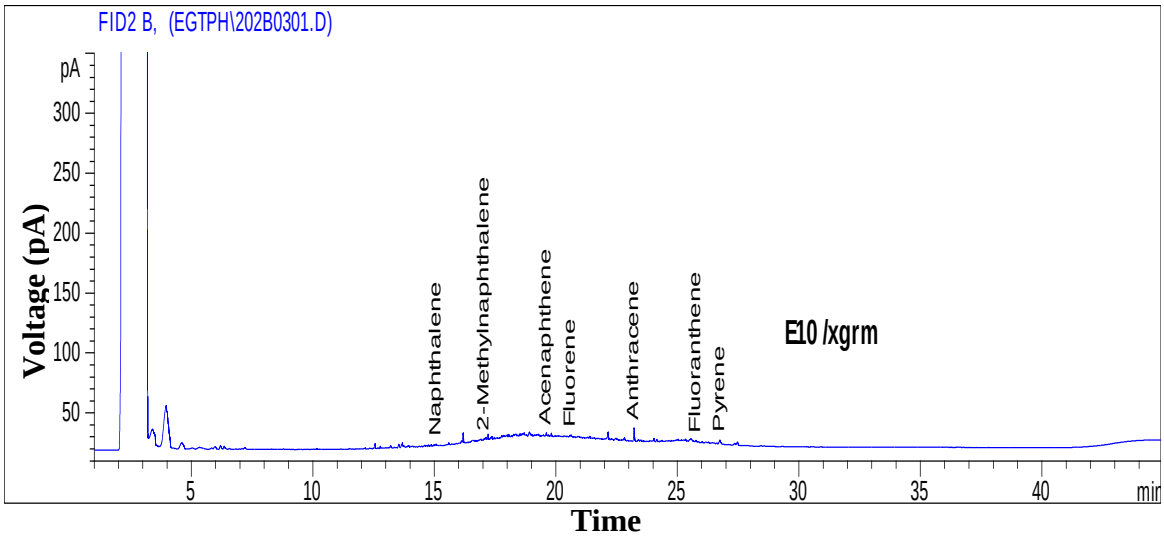

Figure 29: Chromatogram of aromatic hydrocarbon content in 100% polluted soil supplemented with 20g ash after plant harvest

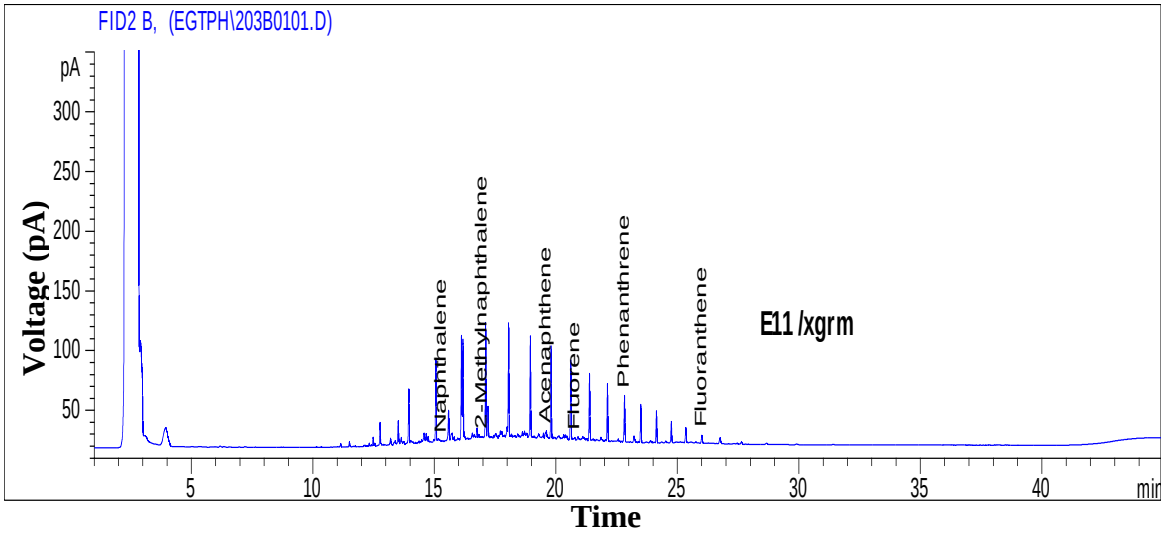

Figure 30: Chromatogram of aromatic hydrocarbon content in 100% polluted soil without ash and plant left under field condition for the duration of the study.

DISCUSSION

Oil spills may affect plants by retarding seed germination, decreasing plant height, stem density, photosynthetic rate, and biomass, or causing complete mortality (Lin and Mendelssohn, 2008). The intensity of oil damage depends upon a number of abiotic and biotic factors primarily, including the type and volume of spilled oil, the species and extent of plant coverage, the season of the spill, prevailing weather conditions, degree of oil weathering, and soil composition (Lin and Mendelssohn, 2009). In this study, the vegetative data recorded for plants grown indicated a reduction or growth retardation in crude oil polluted soil supplemented with wood ash. Germination of C. *aestuans* was higher in unpolluted soil than in polluted soil. One significant observation was the ability of C. *aestuans* to germinate in all concentrations of polluted soil used in this study (Table 2).The difference in percent germination values obtained for unpolluted and polluted soil samples was significant. The result suggests higher values for percent germination of seeds sown in soils supplemented with 20g wood ash fourteen days after sowing seeds. The germination of *L. siceraria* in this study did not show a particular pattern. A conspicuous observation was the high percent germination values observed for 50% polluted soil supplemented with wood ash (Table 3). As previously observed too, seeds of *L. siceraria* germinated in all soil samples used in this study. *C. aestuans* and *L. siceraria* exhibited good tolerance capacity in crude oil polluted soil.

During seed germination, plants are particularly sensitive to environmental stress (Ungar, 1996). Chaineau *et al.* (1997) suggested a link between poor germination and subsequent poor growth of plants in hydrocarbon – contaminated soils. On the otherhand, Li *et al*. (1997) remarked that germination of seeds could be unaffected whereas growth is diminished significantly in hydrocarbon contaminated soils. Crude oil in soil makes that soil to become hydrophobic and this condition creates dehydration effects on plants growing in the soil (Vwioko and Fashemi, 2005; Anoliefo and Vwioko, 1995). The supplementation of polluted soil with wood ash was designed to tackle two challenges plants contend with when growing in polluted soil. One was to reverse the hydrophobic soil condition of polluted soil. The other was to provide nutrients like N, P, K, and Ca that may be limiting in the polluted soil. The wood ash supplementation to polluted soil made the soil to become wettable; reversing the hydrophobic to hydrophilic condition. Water could percolate the soil matrix after mixing with wood ash. The seeds and seedlings did not have to contend with dehydration often created by oil in soil. In this study, the seeds of *C.aestuans* and *L. siceraria* did not contend with soil hydrophobicity. The poor germination observed in some test soil samples were not direct effects of hydrophobic condition. This hydrophobic condition is connected to delayed seed germination, which was not the obvious case in this study. Six days after planting (DAP), seed germination has been recorded for all soil treatments used in this study.

The inhibition of plant growth in crude oil polluted soil were evident from the data recorded for plant height of *C.aestuans* and vine length and leaf elongation rate of *L. siceraria* (Figures 1, 2, 3, and 4). The wood ash promoted growth of *L. siceraria* in uncontaminated soil. This is attributed to the nutrients contributed by the presence of wood ash in the soil used. Interestingly, the mean values for vine length of plants grown in 50% crude oil polluted soil supplemented with either 10g or 20g ash were higher than the mean values for plants in uncontaminated soil without ash. Growth was promoted by as much as 22.00% in 50% polluted soil. Plants grown in the 100% crude oil polluted soil did not respond in that wise. Many authors have suggested that nutrient availability and deficiencies were challenges of plants grown in oil polluted soil (Adam and Duncan, 2002; Baker, 1970; Chaineau *et al*., 1997; Cunningham *et al*., 1996; Isirimah *et al*., 1989;; Kirk *et al*., 2002). The growth data obtained in this study showed that nutrients supplied as contained in wood ash were insufficient to promote good growth of plants in crude oil polluted soil. Shirdam *et al*. (2008) reported the performance of sorghum (*Sorghum bicolor*) and common flax (*Linum usitatissumum*) in crude oil polluted soil supplemented with peat fertilizer, humus and animal fertilizer. Of the three supplements in crude oil polluted soil, only peat fertilizer gave a considerable performance in shoot biomass reduction. Their conclusion was that the inhibitions in plant biomass and shoot height were direct effect of toxic compounds in the petroleum hydrocarbons to the plants.

Our interest to identify crude oil components in soil that were partially degraded and /or undegraded after plant growth led us to carry out the GC analysis of soil samples after plant growth. The chromatograms obtained from the GC analysis showed differences in crude oil components detected in various soil samples. The chromatograms of unpolluted and polluted soil samples obtained from field are shown in Figures 5-8. These chromatograms indicated the presence of naphthalene, 2-methylnaphthalene, acenaphthalene, fluorene, phenathrene, anthracene, among others as the polycyclic aromatic hydrocarbons identified (Figures 5 & 6). The peaks were more and visibly higher for polluted soil. Clearly, there were more components of crude oil detected. Among the aliphatic hydrocarbons detected were nonane, decane, dodecane, eicosane, hexacosane,octacosane, triacosane,etc, (Figures 7 & 8). The peaks of these components were very conspicuous in polluted soil.

The chromatograms obtained for soil analysed after plant harvest showed some interesting observations. Wood ash supplementation (10g and 20g) in unpolluted soil with plant growth resulted in complete degradation or metabolism of both aromatic and aliphatic hydrocarbons initially detected (Figures 9, 10, 20 and 21) in unpolluted soil obtained from the field. Also, ash supplementation in 50% and 100% polluted soil with plant growth and no plant growth showed reduction in concentration of aliphatic and aromatic hydrocarbons in soil when compared with the initial polluted soil sample obtained from the field. One important observation with 50% polluted soil, wood ash supplemented soil without plant growth gave a complete degradation of aliphatic hydrocarbon except nonane, docosane and tetracosane; naphthalene, 2-methyl naphthalene, acenaphthalene, acenaphthene, fluorene and anthracene for aromatic hydrocarbons (compare Figures 11, 13, 22 and 24). Ten grammes wood ash in 50% polluted soil provided better platform for degradation of crude oil components than 20g ash. The growth of plants in 50% polluted soil supplemented with wood ash (10g and 20g) produced chromatograms with more peaks of crude oil components detected (see Figures 12, 14, 23 and 25).

The contribution of plant seems to have affected the metabolism of aliphatic hydrocarbons and facilitated aromatic hydrocarbons degradation using the total values obtained in the analysis (values not shown here). GC analysis of 100% polluted soil supplemented with 10g and 20g ash showed that 20g ash supplementation with plant growth resulted in degradation of crude oil components indicated by very reduced peaks in the chromatograms obtained for aliphatic hydrocarbons (see Figures 15, 16, 17, 18 and 19). The growth of plants in 100% polluted supplemented with 10g or 20g ash showed that aromatic hydrocarbons were degraded more in the presence of plants and 20g ash supplementation provided a better condition for degradation (see Figures 26,27,28 and 29). The presence of higher peaks showed by the chromatograms of soil media without plants indicated that plants contributed to the degradation processes in soil media where they were present. A significant conclusion from this study is that wood ash supplementation in crude oil polluted soil facilitated the degradation of polynuclear aromatic hydrocarbon components.

Cervamtes-Gonzalez *et al*. (2009) stated that available reports indicated that hydrocarbon removal regularly occurs on a specific group of hydrocarbons; specificity occurs due to the capacity of the involved microorganisms and according to its degrading enzyme system (Leblond *et al*., 2001), including also the chemical nature of he hydrocarbons. Shailubhai *et al*. (1984) used a *Rhodotorula* sp. to treat oil sludge and found that the susceptibility to degradation was in the following order: saturate fractions > aromatic fractions >asphaltic fractions. Other authors reported the enhancement over a specific hydrocarbon fraction due to the addition of carbon sources, such as Namkong *et al*. (2002), reported a preferential degradation of n-alkanes over other hydrocarbons in diesel contaminated soil by the addition of sewage sludge or compost as an amendment for supplementing organic matter, regardless of the kind nor the amount of organic amendments.

Enhanced natural attenuation (ENA) and bioaugmentation are two bioremediation strategies that have been applied for the remediation of contaminated soil. ENA stimulates the metabolic activity of the indigenous soil microflora through the addition of nutrients or alternative carbon sources. In this study, the addition of wood ash as a soil supplement was to enhance the growth of plants and the degradation capability of indigenous soil microflora. The reduced growth of plants in polluted soil indicates sensitive response of plants to chemical substances in soil. Plants that are able to grow in contaminated sites take up long chain (heavy) alkanes into their roots rapidly and slowly translocate them into stems and leaves as a result of their low solubility in water (Palmouth *et al*., 2002). Additional explanation suggested include the effect of small aliphatic, aromatic, naphthalic and phenolic compounds in crude oil that may reduce respiration, transpiration, photosynthesis and hormonal stress response (Vouillamoz and Milke, 2001; Trapp *et al*., 2005).

These effects however vary with individual plant species and their physiological responses to contaminants. The continued growth of a plant in the presence of a contaminant suggests that the plant is a potential phytoremediant. The mechanism believed to be responsible for most of the degradation of petroleum hydrocarbons in vegetated soil is the stimulation of growth and activity of degrading microorganisms in the rhizosphere (Frick *et al*., 1999). Wood ash offered additional nutrients to soil microflora enhancing hydrocarbon degradation. Wood ash supported the degradation of aliphatic and polynuclear aromatic hydrocarbon fractions by indigenous soil microflora. Cervantes-Gonzalez *et al*., (2009) stated that in the presence of keratinous waste (ground chicken feathers) solely the biodegradation of aliphatic fraction of crude oil hydrocarbon was significantly increased and remarked further that a preferential degradation of the C_{18} to C_{28} aliphatic compounds were observed. Wood ash gives an advantage over this. It is important to state that wood ash improved crude oil removal from contaminated soil. The challenge in applying accelerated biodegradation technique is not the ability to stimulate bacteria to degrade hydrocarbons; it is judging how low the hydrocarbon concentration can go. In this study, the estimated concentrations of different components of crude oil contaminated soils were insignificant (compare Figures 29 and 30, for example).

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