Chapter 6

Effectiveness of Sorghum Husk and Chicken Manure in Bioremediation of Crude Oil Contaminated Soil

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Additional information is available at the end of the chapter

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Abstract

This study identifies total petroleum hydrocarbons (TPH) and traces of heavy metals such as zinc, lead, cadmium, nickel, and copper in crude oil-contaminated soil. It also focuses on the use of poultry manure and sorghum husk in the bioremediation of the contaminated soil. Crude oil-contaminated soil sample was divided into five parts (A: untreated, B: poultry manure, C and D: poultry manure and sorghum husk in ratios 1:1 and 3:1, respectively, and E: sorghum husk). The heavy metals concentrations and TPH content were assessed initially in the untreated soil sample and later on the 5th, 10th, 15th, 20th, 25th, and 30th days after adding the stimulants. Gas chromatography-mass spectroscopy (GC-MS), atomic absorption spectrometer (AAS), pH and conductivity meters were used for TPH, heavy metals, pH and electrical conductivity analyses, respectively. The results showed soil sample C to have highest TPH reduction, while the soil sample E exhibited 96.1% reduction in nickel, 97.5% reduction in zinc, 100% reduction in lead, and 99.3% reduction in copper. The pH of the soil ranged from 7.13 to 7.92 (within the range 6.5–8 suitable for microbial growth). The electrical conductivity for soil samples B–E increased and also in the acceptable range of 130–2320 μS/cm.

Keywords: bioremediation, crude oil, contaminated soil, sorghum husk, poultry manure

1. Introduction

Bioremediation is a cost effective and environment friendly method, which involves the use of microorganisms to degrade contaminants from soil and water. It is the use of naturally cropped up microorganisms by humans in an attempt to decontaminate man-made contaminants. The advantage of this technique is that it is conservative of soil characteristic and

texture, but the success of its application is dependent on the nature of the contaminant in the soil and environmental conditions under which the test will be carried out. Because bioremediation utilizes microbial activity, it is therefore necessary to provide nutrients, oxygen, and suitable temperature to maximize growth. It has been reported that the use of organic waste enhances bioremediation of crude oil-polluted soil by facilitating soil aeration and raising the water holding ability of the soil [1].

Bioremediation promotes natural clean-up of dangerous toxins by the environment and can either be done in-situ or ex-situ. In-situ bioremediation is carried out at the site of interest whereas in ex-situ bioremediation, the contaminated soil is collected and processed at an offsite area such as a laboratory for cleansing [2]. The main procedure frequently used in oil-spill bioremediation technique is biostimulation, that is, through the addition of nutrients (nitrogen, phosphorus, and potassium) and regulation of environmental conditions to maximize growth of native bacteria and biological degradation. Thus, bioremediation can be said to be a method, which utilizes microbial diversity and metabolic flexibility to convert chemical contaminants into less toxic products [3]. Various studies have investigated the part of microorganisms and bulking agents such as rice husk in the remediation of oil-contaminated soils [4, 5].

A research by Hidayah and Mangkoedihardjo [5] on remediation of chromium-contaminated soil using rice husk showed that microbial growth of indigenous bacteria increased by three folds with a corresponding fall in chromium levels in the first 2 weeks. The treatments adopted comprised of soil and rice husk in varied ratios. The results proved that rice husk helps in biostimulation and has a significant role in improving bioremediation of chromium-polluted soil [5]. Additionally, the study by Adams et al. [4] on the use of rice husk, chicken manure, and their combination for bioremediation of crude oil-contaminated soil revealed that rice husk removed more total petroleum hydrocarbons (TPH) compared to the chicken manure and their combination.

Other agricultural waste that has been used for crude oil-contaminated soil bioremediation includes melon husk [6] and peanut hulls [7]. Sorghum husk moreover is also known to contain some nutrients that can enhance microbial growth and also adsorb heavy metals. Red sorghum husk is a rich source of anthocyanins, which are plant pigments known to increase microbial activity. It is low in protein and ash content but rich in fibrous materials.

Ogboghodo et al. [8] stated that the addition of poultry manure to crude oil-polluted soil caused an increase in plant height from 20 to 149 cm as level of manure applied increased from 0 to 150 kg/ha and dry matter yield also increased from 27 to 58 g within 2 weeks of study, and proposed the adoption of poultry manure for the stimulation of hydrocarbon in the soil as a good technique of battling petroleum contamination in the natural environment. Another study by Okafor et al. [9] has proven that poultry manure is rich in organic matter thus allows for the growth of a substantial amount of microorganisms. Varied concentrations of poultry manure were studied and a positive correlation between growth of microorganism and increase in poultry droppings was observed. The sample amended with the highest amount of poultry droppings exhibited greatest reduction in TPH. Isolates of the hydrocarbon degrading microorganisms in this study include Bacillus spp., Pseudomonas

spp., Flavobacterium spp., Fusarium spp., and Aspergillus spp., while the total heterotrophic bacterial and fungal counts of the poultry droppings were 4.2×10^4 and 1.8×10^4 cfu/g [9]. No work has been done on the study of the effectiveness of sorghum husk and its combination with poultry manure in bioremediation of contaminated soil. Thus, this present work intends to study the effectiveness of the sorghum husk, poultry manure, and their combination in bioremediation of crude oil-contaminated soil from the Niger-Delta Nigeria.

2. Materials and methods

2.1. Materials

The crude oil-contaminated soil used in this study was obtained from the Niger-Delta region of Nigeria. A total 15 plates (5 samples in triplicates; see **Table 1**) were used as simulated environment, while, pH meter, conductivity meter, GC-MS, and atomic absorption spectrometry (AAS) were used to determine the soil pH, electrical conductivity, TPH, and heavy metals, respectively. Other devices used were electronic weighing balance conical flasks, beakers, spatula, and stirrers. Hexane was used as solvent for the extraction purpose for all the analyses that were carried out.

2.2. Soil samples preparation

The soil samples were prepared for bioremediation by removing sticks and stones after which the different stimulants (sorghum husk, chicken manure, and their mixtures) were then added in varied amounts to enhance hydrocarbon degradation and successful microbial growth. About 200 g of the crude oil-contaminated soil was weighed into each of the plastic containers and labeled accordingly (see **Table 1**) in triplicates. The soil sample used was divided into five parts (A: untreated, B: poultry manure only, C and D: poultry manure and sorghum husk in ratios 1:1 and 3:1, respectively, and E: sorghum husk only). In order to avoid low oxygen and preparing aerobic soil conditions, the soil samples were mixed twice per week for a period of 30 days. The soil samples were kept under a controlled temperature of 29°C and their pH were kept in a range of 6.5–8.5, which is the optimal range for microbial growth and reproducibility [4].

Soil samples	Description
A	Contaminated soil only
B	Contaminated soil mixed with poultry manure only (8/200 g-soil)
C	Contaminated soil mixed with poultry manure and sorghum husk in the ratio 1:1 (16/200 g-soil)
D	Contaminated soil mixed with poultry manure and sorghum husk in the ratio 3:1 (32/200 g-soil)
E	Contaminated soil mixed with sorghum husk only (8/200 g-soil)

Table 1. Soil sample preparations.

2.3. pH and conductivity

The samples for pH and conductivity determination were prepared by adding 5 g of the soil sample in 10 ml deionized water. The mixture was stirred and kept for 30 minutes before the conductivity and pH soil were determined. The first values were obtained on the 5th day of the study and thus served as the initial pH of the soil samples.

2.4. Total petroleum hydrocarbon and heavy metals determination

An extraction process was carried out by putting 20 g of soil sample in 20 ml of hexane and shaking it mechanically for 30 minutes. The mixture was then placed in a centrifuge to separate the liquid component from the solid component. The liquid component was obtained from the mixture by filtration and the filtrates were analyzed using GC-MS (this process was repeated every 5 days for 15 days) and AAS for TPH and heavy metal reduction, respectively. The heavy metals in the soil samples were determined after the 30 days of study.

2.5. Total organic carbon (TOC)

There are broad ranges of organic carbon in soil from the breakdown of either plants or animals [10]. TOC is useful in assessing quantity of organic matter in a soil sample. The Walkley-Black chromic acid wet oxidation method as described by Resources [11] was used in determining the TOC in the soil samples.

$$\text{TOC}(\%) = \frac{0.003\ \text{g} \times \text{N} \times 10\ \text{ml} \times (1 - T/S) \times 100}{\text{ODW}} \quad (1)$$

where N = concentration of $K_2Cr_2O_7$ solution, T = Volume of $FeSO_4$ used in sample titration (mL), S = Volume of $FeSO_4$ used in blank titration (mL), ODW = Oven-dry sample weight (g).

3. Results and discussion

3.1. pH and conductivity

The pH of the soil is a significant soil parameter. **Figure 1** shows the changes in the pH values for all the soil samples with time. The pH values were increasing and decreasing in an irregular manner. Soil pH can be highly variable depending on the type of soil and location of the soil. The soil pH ranges from 2.5 in mine spoils to 11 in alkaline deserts [12]. Extreme pH of soils would have a negative influence on the ability of microbial populations to degrade hydrocarbons. Soil sample A showed the highest pH values from the 5 to 20th day (7.55, 7.56, 7.70, and 7.61, respectively), while the soil sample B exhibited the lowest pH value on the 5th day, and E on the 10th, 15th, and 20th day, respectively. The pH values of the samples containing stimulants (samples B–E) had lower pH values compared to the control sample (sample A) in the first 20 days of the study. However, on the 25th and 30th day, sample B exhibited the highest pH value contrary to its pH (lowest) in the first 5 days of the study.

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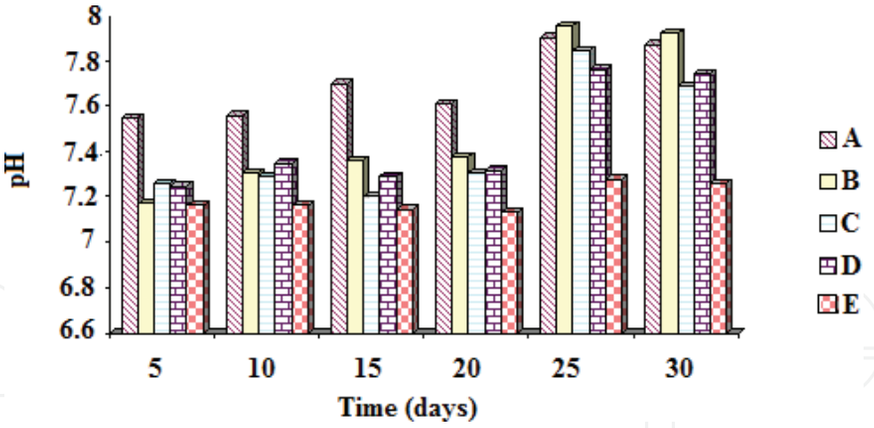

Figure 1. Change in pH with time.

Addition of water after day 10 caused a rapid increase in the pH values for samples A and B, while others decreased. However, pH value continued to increase till the end of the study for all the soil samples. The change in pH could be attributed to increase in metabolic activities of microbes and the degradation of hydrocarbons in the soil that may have led to the synthesis of intermediary metabolites [13]. It is important to note that the pH was within proper range (6–8) for microbial growth throughout the treatment process [14].

Figure 2 shows the electrical conductivity (EC) results obtained for the soil samples from day 5 to 30 of the bioremediation study. The electric conductivity ranges between 159 and 1533 μS/cm for all the soil samples. It was observed that the conductivity initially increased in the first 15 days before decreasing in the last 20–30 days. The soil sample B showed highest electrical conductivity throughout the study period. The soil samples C and D also exhibited higher EC compared to the soil sample E. The control soil sample A had the lowest EC throughout the study period. The increase can be due to the presence of nutrients at the beginning of the treatment, which was gradually decreased with time by microbes leading to the observed decrease.

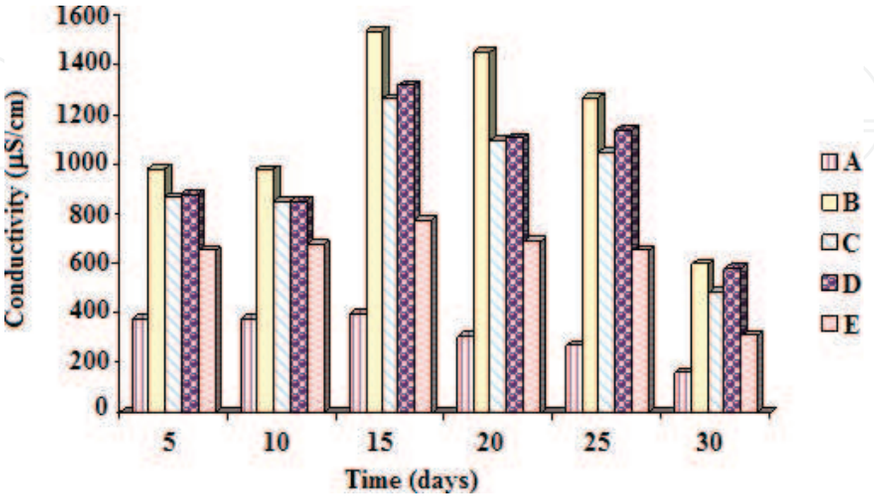

Figure 2. Change in electric conductivity with time.

Correlating the pH with the EC results (**Figures 1** and **2**) showed that at the first 15 days of the study, the pH values were generally lower with higher EC values. Also, at the last 15 days, higher values of pH were observed with lower EC values. These results indicate higher microbial activities in the last 15 days and possible higher TPH degradation, which lowered the EC of the soil samples thereby increasing the pH values. It is known that EC depends on the concentration of all the ions present in the soil samples. The higher the concentration of the ions in the soil, the stronger or higher the EC values for the soil. Soil that exhibits high alkalinity will have less soluble salt in it [15], which indicates that at low soil pH value, high soluble salt content is present in the soil. Thus, the soil will have high EC.

3.2. Heavy metals

The concentration of heavy metals in the soil samples was determined using AAS. **Table 2** shows the percentage removal of heavy metals from the crude oil-contaminated soil. The initial contaminated soil had traces of copper, nickel, zinc, and lead (0.1840, 0.0820, 0.4120, and 0.0198 mg/g, respectively). A significant reduction in the amount of heavy metals in all the soil samples was observed after a 30-day bioremediation study. The decrease in the heavy metal in soil sample A could be due to moisture, aeration, and natural bioremediation due to the natural microbes in the soil sample. Sorghum husk (soil sample E) showed more heavy metal removal efficiency compared to poultry manure (soil sample B) and their mixtures (soil samples C and D). The highest removal of >99% was observed in samples A, C, D, and E for Cu, while the lowest metal removal was for Pb in soil samples A, B, and D. However, Pb was totally removed from soil sample E, which contained sorghum husk only and about 94.9% of Pb was removed from sample C, which contained a mixture of poultry manure and sorghum husk in ratio 1:1.

Metal removal in soil sample A indicates that microorganisms indigenous to that soil sample have greater metal reduction ability than those present in soil samples C, B, and D to which stimulants was added. Nonliving biomass such as rice husks and corn cobs have been proven to be effective in adsorption [4, 5, 16]. The results obtained in this study show that sorghum husk also has high capacity for adsorption of heavy metals due to its high fiber [17, 18].

Heavy metals	A (%)	B (%)	C (%)	D (%)	E (%)
Ni	95.5	94.6	95.1	93.7	96.1
Zn	97.3	96.0	96.8	92.5	97.5
Pb	69.7	82.8	94.9	76.8	100.0
Cu	99.2	95.0	99.3	99.1	99.3
TOC	0.08	0.78	0.70	0.48	0.74

Table 2. Removal ratios of heavy metals from the soil samples and TOC in the soil.

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3.3. Total organic carbon

The results obtained from the total organic carbon analysis are presented in **Table 2**. The TOC of the untreated soil sample was 0.15%. A reduction in TOC is observed only in the control soil sample A. The reduction of TOC in A indicates natural microbial activity in the soil since microorganisms tend to use soil organic carbon as a source of energy. Additionally, the increase in TOC in the other groups can be attributed to the decomposition of the plant and animal wastes that were added.

3.4. Total petroleum hydrocarbon

The results from the GC-MS analysis showing the spectra for all the untreated and the treated soil samples on day 5 and 15 are shown in **Figures 3** and **4** and the major TPH present in the soil samples at those days are presented in **Tables 3** and **4**.

The results showed about 23 TPH present in the crude oil-contaminated soil. However, the control soil sample showed a reduction to about six major TPH on the 5th day and only four major ones on the 15th day (**Table 4**). This could be due to the indigenous microbial in the soil, which have the ability to generally breakdown heavy metals and petroleum hydrocarbons as long as favorable environmental condition is provided for them to carry out their metabolic actions.

The benefits of relying on these microorganisms for biodegradation instead of adding new ones are that they are attuned for survival and growth in that condition. Furthermore, their capacity to use hydrocarbons is dispersed among an assorted microbial populace. These populaces take place in common biological systems and either autonomously or jointly process different hydrocarbons [19, 20].

The results of the control sample show biodegradation of the TPH by natural microorganisms present in the soil. Constant watering and aeration of samples after the 10th day enhanced metabolic microbial activity on the 15th days, but the absence of stimulants in the treatment groups may have contributed to slow degradation of hydrocarbons. Biodegradability of hydrocarbons in soil has shown correlation to their water solubility [17]. This is because bacteria in the unsaturated soil occur mainly in the interstitial water of soil. Therefore, solubility of the chemical will determine its concentration in soil. The control group showed high concentrations of 9,12-octadecadienoic acid, ethyl oleate and tetracosane, which were absent in the original sample. A reduction in the amount of 1-nonadecene is noticed from 12.20 s in the untreated sample to 11.23 s in control group A. As generally observed in the degradation of TPH results, it can be seen that the degradation was faster (15 days). This was due to the high temperature (>30°C) at the time of the study in Yola (North Eastern, Nigeria). Temperature affects the solubility of TPH [21]. Hydrocarbon biodegradation can however take place over a wide ranges of temperature (highest degradation rates usually take place between 30 and 40°C in soil environments, 15–20°C in marine environments, and 20–30°C in some fresh water environments [22, 23].

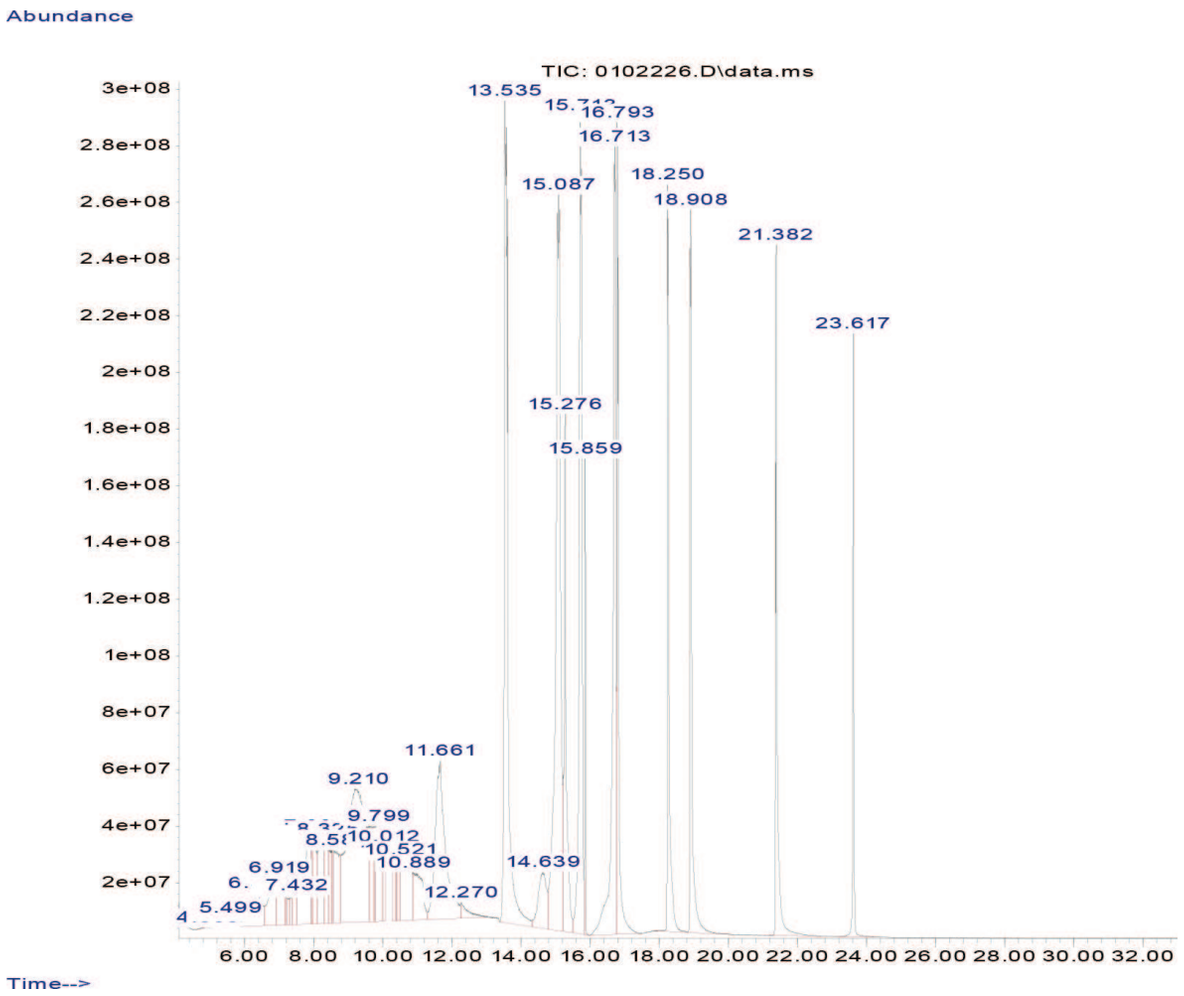

Figure 3. GC-MS spectrum of contaminated soil.

Soil sample B, which contained poultry manure only, showed a great reduction in hydrocarbon content when compared to the initial sample and control group A. However, more TPH were observed on the 5th day in soil sample B. This could be due to high degradation of the TPH by the microorganisms present in the poultry manure. The hydrocarbons heptacosane and oleic acid were present in high concentrations of 58.44 and 40.71, respectively (**Table 4**). Formation of oleic acid serves as the evidence of oxidation. The biodegradation of oil pollutants in soil needs necessary nutrients. Choi et al. [24], Kim et al. [25], and Pelletier et al. [26] studied the effect of fertilizers on crude oil-contaminated soil bioremediation in sub-Antarctic intertidal sediments over a 1 year. The results of the study showed that microbial, chemical, and toxicological parameters demonstrated the use of various fertilizers in a pristine environment. In study using poultry manure as organic fertilizer in contaminated soil increased biodegradation was reported but the extent of biodegradation was influenced by the incorporation of alternate carbon substrates or surfactants [27].

It can also be seen in **Table 4** and **Figure 4**, there is a reduction in the hydrocarbon composition of the soil sample C in comparison to the initial untreated soil sample. Concentrations of 4-heptenal, 1-heptene, octadecane, 1-chloro-acetamide, and methyl 7-oxopentadecanoate were quite low relative to that of octadecanal, heptacosane, hexadecanoic acid, and methyl ester in the

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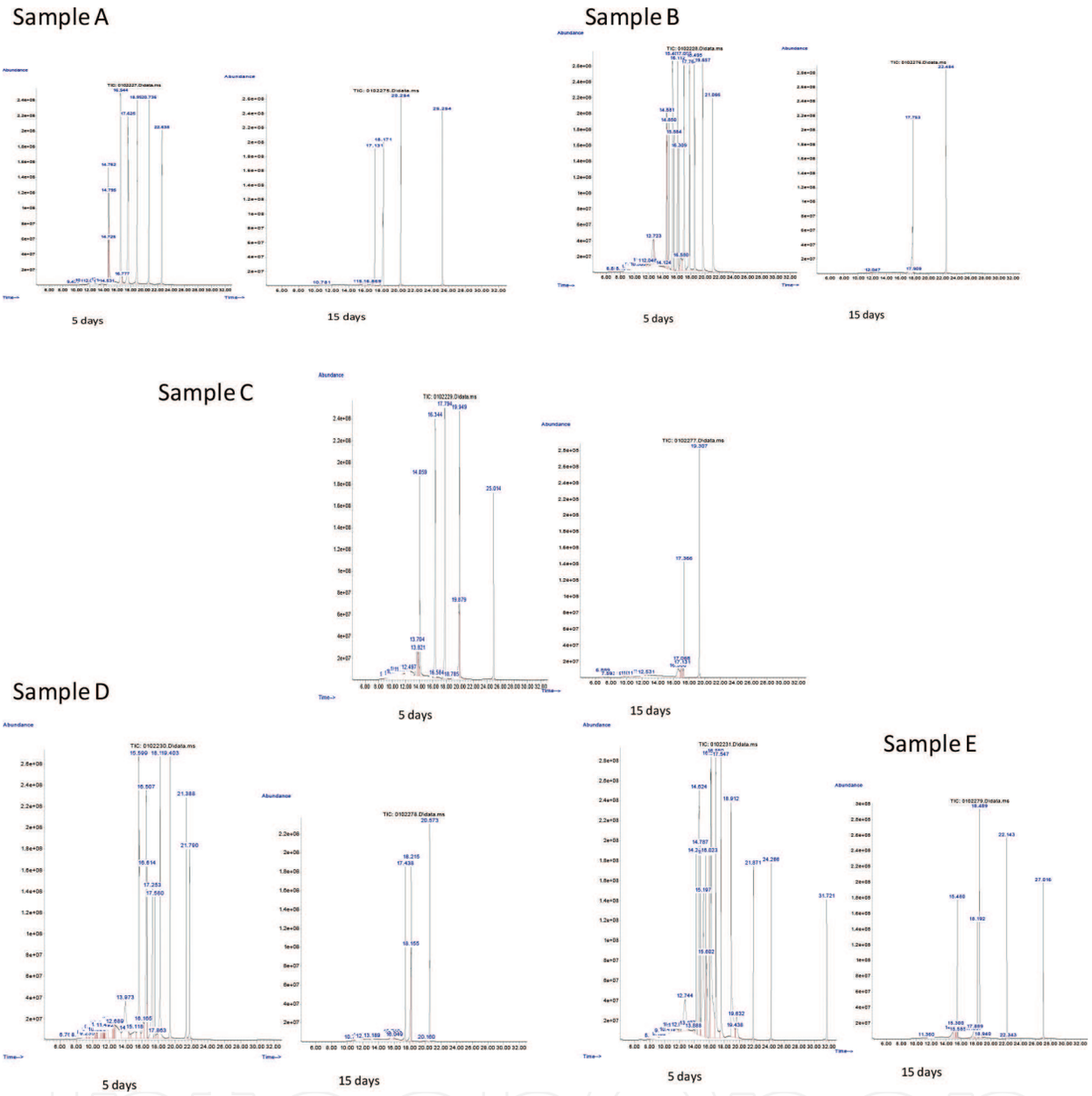

Figure 4. GC-MS spectra of soil samples A–E.

contaminated soil sample. The presence of esters and other oxidized compounds is an indication that the presence of poultry droppings and sorghum husk induced oxidation of the hydrocarbon compounds. The presence of hexadecanoic acid in soil samples C and D is as a result of degradation by sorghum husk (**Table 4**) and oleic acid by the poultry manure. The carbon chains are also shorter ranging from C_7 to C_{20}. The microbial attack on hydrocarbons depends on the type of hydrocarbon. The hydrocarbons susceptibility to microbial attack is ranked in the following order: n-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes [28].

Table 4 shows that treatment of contaminated soil with poultry manure and sorghum husk in a 3:1 ratio (sample D) resulted in a decrease in hydrocarbon components. There was oxidation of some of the compounds from the initial crude oil-contaminated soil analysis to 1-hexanoic acid and 1,2,15-pentadecanetriol.

No	Retention time (min)	Area (%)	Major molecule
1	6.356	1.13	Heptane
2	6.839	1.08	Nonadecanol
3	7.896	2.16	Dodecane
4	7.967	1.16	Oxirane
5	8.266	1.49	Octadecane, 1-Chloro-acetamide
6	8.325	1.03	Cyclohexane
7	8.582	1.45	17-Pentatriacontene
8	9.209	8.41	Hexadecane
9	9.674	1.44	Silane, trichloroeicosylsilane
10	9.800	1.87	Dodecane, 1-Choro-acetamide
11	10.522	2.01	1-Eicosanol
12	10.886	1.44	Cyclohexane
13	11.662	5.46	Pentalene
14	13.536	10.56	Cyclotetradecane
15	14.640	1.56	Cyclohexanol
16	15.088	12.20	1-Nonadecene
17	15.273	3.61	1-Docosanethiol
18	15.715	7.95	Cyclopentadecane
19	16.712	8.60	5-Eicosene
20	16.795	4.26	1-Acetyl-3-(2-pyridyl)-4-phenylpyrazoline
21	18.251	4.19	9-Hexacosene
22	18.908	4.26	Tricosane
23	21.385	3.90	4-Hexenoic acid, 3-Methyl-2,6-dioxo-4-hexenoic acid

Table 3. Major composition of untreated soil sample.

Table 4 and **Figure 4** show a reduction in the number of TPH in the soil sample E compared to that in the initial untreated soil sample. There was an addition of chloride to most of the hydrocarbon compounds in this soil sample; this could be due to the presence of the sorghum husk. The soil sample E showed the highest amount of hydrocarbon compounds in comparison to other treatment groups on the 5th and 15th days. This could be due to the pH (lowest) of the soil sample on these days as compared to the other soil samples. Reports have shown that higher biodegradation is achieved at increased pH values. According to Verstraete et al. [29], adjusting the pH value of gasoline from more acidic (4.5) to neutral (7.4) doubled its rate of biodegradation. Meanwhile, the rates decreased significantly when the pH was raised again to 8.5. Also, the optimum pH recorded by Dibble and Bartha [30] for the mineralization of oily sludge in soil ranged from 5.0 to 7.8.

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Samples	Days	Retention time (min)	Area (%)	Main probable molecules
A	5	14.762	3.63	11-Hexacosyne
		14.795	7.56	Ergosta-8,14-dien-3-ol
		16.544	25.43	Oxirane
		17.625	25.27	1-Octadecene
		18.991	16.12	Oxirane
		20.736	9.48	Octadecane, 1-(ethenyloxy)-octadecane
		22.638	6.96	Ethyl Oleate
	15	15.661	1.32	Hexane
		15.894	1.45	Undecane
		15.983	1.83	3,3′-Oxybis-azocine
		17.129	11.23	1-Nonadecene
		18.174	37.38	9,12-Octadecadienoic acid
		20.257	22.32	Ethyl Oleate
		25.253	23.69	Tetracosane
B	5	14.533	5.65	Cyclohexadecane
		15.404	13.95	1-Nonadecene
		16.115	13.32	Oxirane
		17.763	11.02	Ethyl Oleate
		18.496	5.35	2-Bromo-6-methyl heptane
		19.654	6.64	Tetracosane
		21.069	6.64	15-Tetracosenoic acid, Methyl ester
	15	17.756	58.44	Heptacosane
		22.483	40.71	Oleic acid
C	5	13.821	2.64	Tridecane
		14.062	9.25	9-Undecen-2-one
		16.344	27.95	Oxirane
		17.794	23.48	1-Octadecene
		19.879	6.73	9-Octadecenoic acid, Methyl ester
		19.949	14.29	9-Hexacosene
		25.014	6.25	Phemetrazine
	15	6.654	1.58	4-Heptenal
		6.887	1.78	1-Heptene
		16.586	14.13	Octadecanal
		16.986	7.83	Oleic acid
		17.070	5.85	Octadecane, 1-Chloro-acetamide
		17.129	5.66	Methyl 7-oxopentadecanoate
		17.368	12.92	Heptacosane
		19.308	43.48	Hexadecanoic acid, Methyl ester

Samples	Days	Retention time (min)	Area (%)	Main probable molecules
D	5	13.972	7.69	10-Octadecenal
		15.601	17.87	3-Eicosene
		16.507	14.51	1-Octadecene
		17.254	10.28	1-Docosene
		18.165	9.98	9-Octadecene
		19.403	5.51	1-Nonadecene
		21.790	3.90	Heneicosane
	15	15.714	4.69	1-hexanoic acid
		16.049	1.00	1,2,15-Pentadecanetriol
		17.439	16.63	Tetracosane
		18.156	31.11	Eicosane
		18.215	12.73	Tricosane
		20.573	30.98	9-Hexacosene
E	5	12.744	4.75	10-Octadecenal
		14.624	11.31	1-Nonadecene
		15.197	12.71	Hexadecanoic acid, Methyl ester
		15.603	4.87	Pentadecanoic acid, Methyl ester
		16.198	21.27	Heneicosane
		16.850	9.27	12-Octadecenoic acid, Methyl ester
		18.912	9.70	9-Hexacosene
		21.871	2.08	Hexadecanol
		24.266	1.96	Heptacosane
		31.721	1.72	Eicosane, 10-Heptyl-10-octyl
	15	15.480	10.13	Hexacosane
		17.889	2.33	Hexadecane, 1-Chloro-acetamide
		18.192	6.60	Tetracosane
		18.489	24.66	8-Heptadecene
		22.143	17.35	9-Octadecenoic acid, Methyl ester
		27.016	14.30	[1,2′-Binaphthalene]-5,5′,8,8′-tetrone, Tetracosane, Pentacosane

Table 4. Major TPH in soil samples A–E on the 5th and 15th days.

4. Conclusions

There was noticeable reduction in the amounts of Zn, Pb, Ni, and Cu in the soil samples. Sorghum husk treatment was the most effective in reducing the concentrations of the heavy metals as there

was total removal of lead using sorghum husk and 97.5, 96.1, and 99.3% removal of Zn, Ni, and Cu, respectively. There was also relatively high reduction in heavy metals in the control soil sample. Total petroleum hydrocarbon content of soil was analyzed using GC-MS, 10 and 15 days following treatment. There was reduction in the amount of TPH present after 10 days, further reduction was observed after 15 days. The emergence of various oxidized hydrocarbon compounds such as esters, aldehydes, and carboxylic acids is proof of biodegradation of petroleum hydrocarbons by microorganisms present in the poultry manure and sorghum husk. There was also the presence of amine groups in small amounts, which could be from the addition of the poultry manure. The poultry manure and sorghum husk treatment (1:1) seem to be the most effective in reducing soil TPH. The carbon chain length of the compounds in this group was shorter and majority of them were oxidized. Control group A also exhibited high TPH removal indicating that the provision of a suitable environment for microbial growth is just enough to initiate rapid bioremediation.

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