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Research Article

Enhancing the Phytoremediation of Hydrocarbon-Contaminated Soils in the Sudd Wetlands, South Sudan, Using Organic Manure

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Phytoremediation of hydrocarbon-contaminated soils is a challenging process. In an effort to enhance phytoremediation, soil was artificially contaminated with known concentration of light crude oil containing Total petroleum hydrocarbon (TPH) at a concentration of 75 gkg^{-1} soil. The contaminated soil was subjected to phytoremediation trial using four plant species (*Oryza longistaminata, Sorghum arundinaceum, Tithonia diversifolia*, and *Hyparrhenia rufa*) plus no plant used as control for natural attenuation. These phytoremediators were amended with concentrations (0, 5 and 10 gkg^{-1} soil) of organic manure (cow dung). Results at 120 days after planting, showed that application of manure at concentrations of 5 and 10 gkg^{-1} soil combined with an efficient phytoremediator can significantly enhance reduction of TPH compared to natural attenuation or use of either manure or a phytoremediator alone ($p < 0.05$). The study also showed that a treatment combination of manure 5 gkg^{-1} soil, with a phytoremediator gives a similar mean percentage reduction of TPH as manure 10 gkg^{-1} soil ($p > 0.05$). Therefore, the study concludes that use of phytoremediators and manure 5 gkg^{-1} soil could promote the restoration of TPH contaminated-soils in the Sudd region of South Sudan.

1. Introduction

Crude oil activities often lead to changes in the functioning of the soil ecosystem [1, 2]. The crude oil products contaminate soil leading to deficiency of the much needed nutrients for normal functioning of plants [1, 3]. Studies such as [3, 4] have provided proof that crude oil contaminated soils have less content of nitrogen and phosphorus. Besides, the water repellant properties interrupt water infiltration into the soil [5], leading to water and nutrient deficiencies. These adversely affect plant growth and microbial populations such that where oil toxicity persists, and the soil becomes unsuitable for plant growth [4].

Over the years, in oil rich and exploiting countries, efforts have increasingly been taken to remediate contaminated sites [6]. Different approaches; physical, chemical, and biological have been undertaken. However, some of these are expensive while others have harmed the environment, particularly soil health and human livelihoods [3, 7, 8]. For example, excavation (physical approach) has logistics and transport constraints [9–12] while incineration (chemical approach) adds greenhouse gases in the atmosphere leading to global warming. This leaves use of biological approaches such as phytoremediation as the safest, feasible, and desirable [13]. Phytoremediation uses plants and microbes [13]. However, as indicated earlier, contaminated soils have deficient nutrients. To correct this defect, addition of supplementary nutrients such as organic manure is necessary [14, 15].

The use of organic manure is an environmentally safer option because it releases nutrients at a slower rate and as well act as a soil conditioner [4, 8, 16]. Also, organic manure contains nitrogen, magnesium, sulphur, phosphorus, and potassium that support plant growth [17–20]. Organic manure improves soil physical and chemical conditions and

further maintains an adequate supply of soil organic matter with high microbial loads [4]. This enables faster degradation of hydrocarbon contaminants [8, 16]. Evidence is provided by Kaimi et al. [21] in their study of rye grass, that addition of compost manure to the soil increases the rate of removal of Petroleum Hydrocarbons (PHCs) while Obasi et al. [22] reported removal of 60–65% of hydrocarbon from soils treated with manure and municipal biowaste compost. Different from these, this study was on biostimulation of phytoremediators using cow dung due to its prevalence in different environments making it almost cost free [23]. In South Sudan, cow dung is locally available owing to large numbers of cattle. According to Catley [24]; an average household in South Sudan owns four cows, Sudd region inclusive.

South Sudan is the third largest oil producing country in Africa after Nigeria and Angola [25]. Crude oil activities in the Sudd region of South Sudan have affected underground herbs and shrubs and as well, destroyed microbial organisms [26–29]. These environmental hazards are expected to worsen with continued crude oil drilling activities in the region [28]. Hence, biostimulation of crude oil contaminated soil in the Sudd with cow dung is necessary, given its availability. The usefulness of cow dung and other biostimulants have been previously reported. In Nigeria, Essien et al. [30] using *Eleusine indica* established that augmenting crude oil polluted soils with cow dung enhances its remediation potential leading to restoration of polluted soils. Njoku et al. [31] found the same effect using *Glycine max*; Isaac [32] used *Panicum maximum* and *Talinum triangulare*; and Oyedele and Amoo [33] confirmed similar results using Maize plant while Omara et al. [34] used *Sorghum Bicolor* L. (Moench) in petroleum adulterated soils from an automobile repair workshop in Kampala city, Uganda.

The Sudd Region, South Sudan, has a double advantage for benefiting from this innovation, given the prevalence of cow dung [24], and abundance of excellent phytoremediators. Ruley et al. [35] established *Oryza longistaminata, Sorghum arundinaceum, Tithonia diversifolia*, and *Hyparrhenia rufa* as very important phytoremediators in the Sudd wetland. Also, an earlier study by Ruley et al. [28] established their abundance in region. In the 2019 study, Ruley and colleagues observed that these plant species reduced TPH in the contaminated soil by over 50% in the concentration TPH 75 gkg^{-1} soil. Despite the excellence of these phytoremediators (such as *H. rufa)* confirmed by Ruley et al. [35] and the availability of cow dung in the region, no studies have assessed the potential of augmenting crude oil contaminated soils planted with the abovementioned phytoremediators with cow dung. Thus, the objective of this study was to determine the optimal concentration of cow dung capable of enhancing the phytoremediation of hydrocarbon contaminated soils by these phytoremediators in the Sudd region, South Sudan.

2. Material and Methods

2.1. Experimental Site and Design. The study was carried out in a greenhouse subjected to the following treatments; concentrations of cow dung (0, 5, and 10 gkg^{-1} soil) and TPH concentrations of 0 and 75 gkg^{-1} soil. The crude oil (light) used in the experiment was obtained from Dar Petroleum Operating Company Ltd., Operation Base Camp in Paloch, South Sudan. Seeds of four efficient phytoremediators were obtained from the Sudd region of South Sudan (Table 1). To remove TPH under the natural attenuation process, no plants were used (i.e., control). The four selected phytoremediators have high potential for removal of over 50% TPH in contaminated soils assessed in the Sudd region (Table 1).

The trial was conducted in a Completely Randomized Design (CRD) with 30 treatments and replicated three times using Genstat. This gave 90 treatment pots (5 plants × 2 TPH concentrations × 3 concentrations of cow dung × 3 replicates).

Partially decomposed cow dung with a nutrient composition ratio of 1.7 : 0.6 : 0.8 (NPK) was used for the experiment while the soil was collected from uncontaminated natural land in the Sudd region as composite top soil samples at a depth of 0–30 cm. The Sudd region is located within latitudes 60 30′–90 30′ N and longitudes 300 10′–310 45′ E, with an elevation of 320 m above sea level. Table 2 shows the characteristics of the soil sample used.

The soil samples were air-dried and sieved to remove debris and then apportioned into 5 kg per pot for the subsequent experiments. The soil, cow dung, and crude oil were thoroughly mixed on a metallic sheet and then returned into 8-litre pots. The polypropylene pots were perforated at the base to allow drainage and aeration. They were labeled with respective treatments and left to stand for one week before planting. To cater for TPH drainage from the pots, a lid was placed under each perforated pot to collect the leached water. The water was reused to irrigate the pots which controlled TPH loss. Also, periodically, the pot lids were rinsed with deionized distilled water and the resulting wash solution was poured back into the respective pots, further minimizing any losses of TPH.

The seed viability was determined through floatation technique with those remaining at the bottom of the water considered potentially viable. Ten seeds of each plant were sown in each pot and on establishment; the seedlings were thinned to three plants per pot and irrigated with deionized water up to field capacity at two-day intervals up to the end of the experiment (four months after planting). Any weeds that emerged from some of the pots were hand-pulled. Also, the whiteflies were controlled by foliar sprays of Dimethoate (0.05%).

2.2. Data Collection. Data on plant height, total dry matter, and percentage reduction of TPH were collected once at 120 days after planting (DAP). For plant height measurements, the abovementioned ground parts (shoots) of the plants were cut off at the soil surface, followed by destruction of the pots. The carefully crushed pots were shaken into a vat to carefully collect the roots which were washed under running tap water and air-dried to remove surface water. The fresh weights of the partitioned plants

Table 1: Sudd region phytoremediators of petroleum hydrocarbon.

Phytoremediator		Percentage reduction of TPH from concentration of 75 gkg^{-1} soil
Common/local name	Scientific name	
Thatching grass	*Hyparrhenia rufa*	74.40
Wild rice	*Oryza longistaminata*	56.17
False sunflower	*Tithonia diversifolia*	55.92
Sudan grass	*Sorghum arundinaceum*	50.12
No plant (control)	No plant (control)	14.80

Source: Ruley et al. [35].

Table 2: Physical and chemical characteristics of the soil used for the greenhouse experiment.

Parameters	Sand	Clay	Silt	STC	pH	P	TN	SOC	CEC	K	Na	Mg	Ca
Units	(%)				(H_2O)	mg/kg	(%)			(cmol(+)/kg soil)			
Test value	24.2	61.3	14.5	Clay	6.71	15.64	0.27	5.01	26.6	1.69	0.94	1.25	9.92
Critical value[a]	—	—	—	—	5.5	15	0.2	3	25	0.5	<1.0	0.6	10

Source: Ruley et al. [35]. STC: soil texture class; SOC: soil organic carbon; CEC: cation-exchange capacity; [a]critical value according to Okalebo et al. [36] for most crops in East Africa.

were measured. Each of the plant parts was oven-dried at 65°C to constant weight in order to determine the plant total dry matter yield. After removing the roots, the soil from each pot (planted and unplanted) was first homogenized before being sampled and stored at −4°C until further processing and analysis of TPH. The TPH concentrations were determined by extracting 5 g of soil samples with 10 mL dichloromethane (DCM). The extract was then filtered, evaporated, and passed through silica gel before injection to gas chromatograph. The Varian CP-3800 gas chromatograph used was equipped with a flame ionization detector (GC-FID) and split less injector in DB-5 capillary column of 100% polydimethylsiloxane (30 m × 0.25 mm I.D × 0.25 μm film thickness). The carrier gas was helium at a flowing rate of 1.5 mL/min and the injector and detector temperature of 300°C and 320°C. The column head pressure was175 kPa. Oven temperature was programmed at an initial temperature of 35°C, initial hold time of 8°C/min, temperature rate of 27°C/min up to 100°C, temperature rate of 35°C/min up to 300°C, and a final hold time of 5 min. The calibration of GC-FID was performed with the following standards: 2, 10, 25, and 1000 mg/L. TPH was calculated using a programmed integration event timetable. The United States Environmental Protection Agency (USEPA) SW-846 series, method 9071Bd5 was used to compute TPH concentrations. The percentage of TPH degradation was subtracted from the final gas chromatograph results of the soil sample after harvesting and the output was multiplied by 100%. The quality of the batch of soil extracts used was ensured by including blank samples to improve the evaluation of data. The blank samples were prepared using hydrocarbon free soil and then processed using the same extraction technique with known amounts of normal alkanes to check percent recoveries. The hydrocarbon components were analyzed by gas chromatography techniques. The data were displayed as Total Ion Chromatogram (TIC). The information obtained from TIC was used to identify or classify individual components contained in the sample. The reference material was obtained from Petroleum Laboratories, Research and Studies of Sudanese Petroleum Corporation (SPC).

2.3. Statistical Analyses. The data for plant height, total dry weight, and TPH percentage reduction in the soil were analyzed using Genstat to generate treatment means using Fisher's Least Significant Difference (LSD) test at 5% level of significance.

3. Results

3.1. Plant Height and Dry Weight. The mean plant heights for the control with cow dung manure treatments were higher than that of all treatment combinations with TPH concentration 75 gkg^{-1} soil. Generally, and as would be expected, the shortest plants were observed at treatments where there was no manure applied yet the soil was contaminated with TPH concentration 75 gkg^{-1} soil (Table 3). Plant growth (in terms of height) was noticeably improved in treatments containing combinations of TPH concentration 75 gkg^{-1} soil with either application of manure 5 or 10 gkg^{-1} soil. For plant height, no significant difference was observed between treatment combinations of manure 5 and 10 gkg^{-1} soil with TPH concentration 75 gkg^{-1} soil ($p > 0.05$) (Table 3).

The plants in the control yielded more dry weight content than in all treatment combinations with TPH concentration 75 gkg^{-1} soil. Generally, and as would be expected, the light weight plants were observed in treatments where there was no manure applied yet the soil was contaminated with TPH concentration 75 gkg^{-1} soil (Table 4). There were more pronounced reductions in the total plant dry weight in all the four phytoremediators in treatment combination of 75 gkg^{-1} soil manure and TPH concentrations, respectively. This improved with the addition of manure 5 and 10 gkg^{-1} soil to TPH-contaminated soils (75 gkg^{-1} soil) leading to increased biomass yield for all the plant species studied. However, a least significant difference

Table 3: Treatments effect on the height of four phytoremediators.

Phytoremediators (plant species)	TPH, 0 gkg^{-1} soil			TPH, 75 gkg^{-1} soil		
	M, 0 gkg^{-1} soil cm	M, 5 gkg^{-1} soil	M, 10 gkg^{-1} soil	M, 0 gkg^{-1} soil cm	M, 5 gkg^{-1} soil	M, 10 gkg^{-1} soil
H. rufa	160	163	164.4	129	141	142
O. longistaminata	97.1	102.0	102.2	40.1	60.0	62.7
T. diversifolia	116.1	121.2	123.6	90.3	107.2	109.4
S. arundinaceum	130.1	134.3	135.9	70.5	100.5	103.3
CV %			1.9			
LSD (5%)			3.08			

TPH = total petroleum hydrocarbon; M = manure.

Table 4: Treatments effect on the total plant dry weight of four phytoremediators.

Phytoremediators (plant species)	TPH, 0 gkg^{-1} soil			TPH, 75 gkg^{-1}soil		
	M, 0 gkg^{-1} soil (g)	M, 5 gkg^{-1} soil	M, 10 gkg^{-1} soil	M, 0 gkg^{-1} soil (g)	M, 5 gkg^{-1} soil	M, 10 gkg^{-1} soil
H. rufa	13.5	15.1	15.9	7.1	10.1	10.9
O. longistaminata	7.8	8.2	9.2	4.2	6.1	6.7
T. diversifolia	12.1	15.2	16.5	9.0	11.3	12.0
S. arundinaceum	15.5	20.2	20.9	10.1	**12.1**	**13.2**
CV %			2.8			
LSD (5%)			0.9			

TPH = total petroleum hydrocarbon; M = manure.

between treatment combinations of manure 5 and manure 10 gkg^{-1} soil with TPH concentration 75 gkg^{-1} soil was observed in *S. arundinaceum* ($p < 0.05$) while no significant differences were observed in *H. rufa*, *O. Longistaminata*, and *T. diversifolia* ($p > 0.05$) (Table 4).

3.2. Effect of Manure on Phytoremediation of Hydrocarbon Contaminated Soil. The mean percentage reduction of TPH in the four phytoremediators with manure treatments was measured at 120 days after planting (Figure 1). The reductions in the control (i.e., soils with no phytoremediator planted) were lower than those in treatments with 0, 5, and 10 gkg^{-1} soil manure concentrations. Thus, it is evident that the presence of treatments of manure 5 and 10 gkg^{-1} soil improved the percentage reduction of TPH compared to treatments of phytoremediators without manure. No significant differences ($p > 0.05$) in mean percentage reduction of TPH were observed between the manure 5 and 10 gkg^{-1} soil treatments for all the four plant species (Figure 1).

The total ion chromatograms (TICs) of the hydrocarbon fractions from the control and treatments are shown in Figure 2. The chromatograms gave qualitative and semi-quantitative information on the changes in the composition of hydrocarbons in the samples. The compounds in the hydrocarbon fractions of the control ranged from n-C13 to n-C40 and maximizing at n-C26. In the treatment with plant alone, it ranged from n-C20 to n-C40. In both treatments of plant, manure 5 and 10 gkg^{-1} soil, the compounds ranged from n-C29 to n-C32.

4. Discussion

Biostimulation of hydrocarbon contaminated soils with cow dung in the Sudd wetland holds potential of restoring the hydrocarbon contaminated soils through bioremediation. This is boosted by the abundance of *O. longistaminata, S. arundinaceum, T. diversifolia*, and *H. rufa* plant species as naturally existing phytoremediators [28], although their growth is inhibited by high concentration of TPH in the soils. In this study, in the treatments without manure, plant species grew short and recorded light dry weight content compared to the control and treatments with manure. The inhibition is attributed to the unconducive conditions that are caused by crude oil contamination such as water repellency which causes reduced access to water and oxygen by the plants. This partly explains the plant shortness and lightness of the plant dry weight. On the other hand, the crude oil contaminants altered the soil physical properties such as permeability which affected the growth of plant species. The finding is supported by Akinwumi et al. [37] and Nazir [38] who unanimously observed that contamination of soil by crude oil alters the soil physical properties which affects the free flow of total organic carbon and soil mineral nutrients such as potassium, sulfate, phosphate, and nitrate of soil. More light is shed by Akubugwo et al. [39] and Wang et al. [40] that nutrient deficiencies inhibit the growth of plant species in hydrocarbon contaminated soils.

Though Ruley et al. [35] ascertained four plant species (*O. longistaminata, S. arundinaceum, T. diversifolia*, and *H. rufa*) as prominent phytoremediators, this study confirmed that augmentation with cow dung led to a marked improvement in the plant growth characteristics. There were least significant differences between treatments without manure and those with manure in terms of both plant height and dry weight ($p < 0.05$). The improvement in plant height and dry weight content is attributed to the restoration of lost nutrients by addition of cow dung since it contains high nutrient composition hence providing polluted soil with

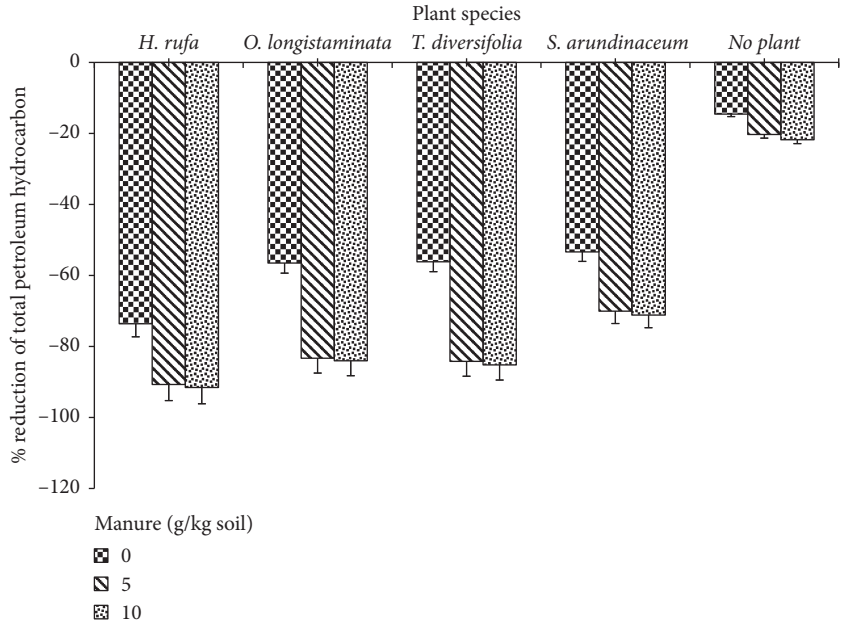
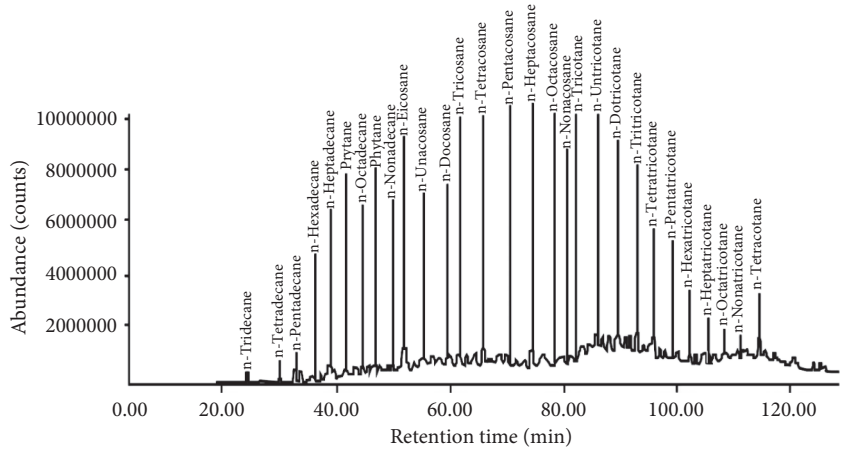
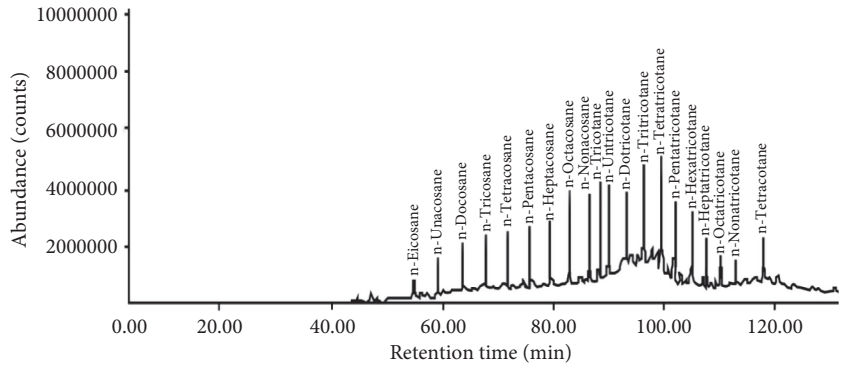


Figure 1: Effect of manure application on phytoremediation of hydrocarbon-contaminated soil; bars show the standard errors (SE) for mean percentage reduction of TPH, $n = 3$.



(a)



(b)

Figure 2: Continued.

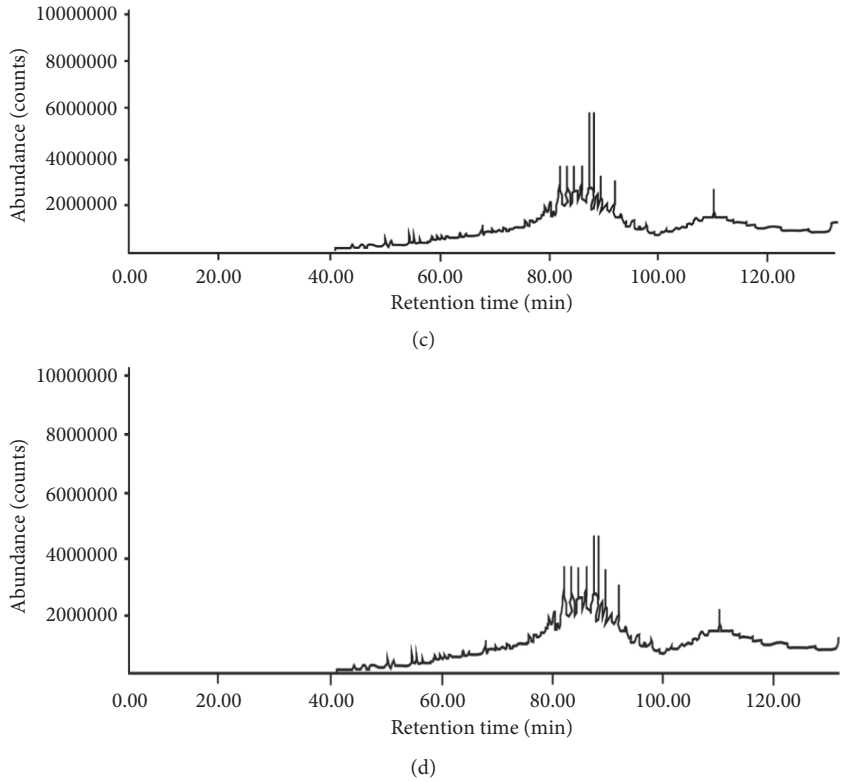


Figure 2: Total ion chromatograms (TIC) of the TPH extracts of the control and treatments during the bioremediation experiment after 120 days. (a) No treatment. (b) Treated with plants. (c) Treated with plant and manure 5 gkg^{-1} soil. (d) Treated with plant and manure 10 gkg^{-1} soil.

nutrient elements. Also, cow dung is effective, economic, and ecofriendly and leads to complete mineralization of hydrocarbons [41] which enhances phytoremediation. Evidence of this effect is provided by the analyses based on TIC which showed a gradual decrease in the hydrocarbon compounds after 120 days implying that some of the compounds had been completely biodegraded and could not be observed in the chromatograms. Basing on this finding, *O. longistaminata, S. arundinaceum, T. diversifolia*, and *H. rufa* become more viable phytoremediators when augmented with cow dung. This assertion rhymes Oyedele and Amoo [33] that addition of cow dung manure improves on the calcium, magnesium, phosphorus, potassium, and nitrogenous contents which are vital elements for better growth of plant species. Essien et al. [30] also observed that the ubiquitous nature of cow dung reduces the cost of using inorganic fertilizers which further reduces the cost of cleaning up crude oil contaminated soils.

In this study, there is a nonsignificant difference between use of manure 5 gkg^{-1} soil and 10 gkg^{-1} soil. The study recommends that any plans should settle for 5 gkg^{-1} soil as the optimal amount. There is a high possibility that using 10 gkg^{-1} soil could divert the attention of the any existing microbes from feeding on crude oil to feeding on nutrients in cow dung. This diversion slows down the process of phytoremediation as was observed in some studies such as Essien et al. [30] that excess application of cow dung has the potential of causing the existing microbes to abandon crude oil and turn to feeding on the nutrients provided by the cow dung.

5. General Conclusion

In different environments, petroleum and petroleum-derived products inhibit plant growth and development. TPH inhibits the normal functioning of plants by interfering with the processes of intake of water and minerals from the substrate. Also, it slows down and impedes a number of metabolic processes from taking place. Case in point, when oil penetrates seed coats, it causes death of the seed embryo. Although remediation of TPH contaminated soils is a challenging task, results of this study have revealed that biostimulating crude oil polluted soils with cow dung significantly enhances plant growth parameters. Consequently, this increases their efficiency as phytoremediators. This study has demonstrated that application of cow dung at concentrations of 5 and 10 gkg^{-1} soil, combined with efficient phytoremediators can significantly enhance the reduction of TPH compared to natural attenuation or use of either manure or phytoremediators alone. Furthermore, this study has elucidated that a combination of organic manure 5 gkg^{-1} soil with a phytoremediator yields the same mean percentage reduction of TPH as 10 gkg^{-1} soil. This study concludes that cow dung manure improves the phytoremediation potential of plant species in the Sudd wetland, South Sudan, and recommends the use of a combination of phytoremediators and 5 gkg^{-1} soil of cow dung as the best combination for enhancing the remediation. Use of cow dung proves cost-effective compared to other remediation techniques and provides an ideal solution to the government

of South Sudan and its development partners for phytoremediation of TPH contaminated soils in and around the Sudd region. Facilitating conditions to support this strategy exist in the region such as the availability of high numbers of cattle, with an average of 4 heads per household. This presents potential opportunities for restoration of crude oil contaminated soils in the region at a low cost.

Data Availability

The data used to support the findings of this study are available on request from Jane Alexander Ruley (janenajeb@yahoo.com; +256756352256)

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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