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# Assessment of plants for phytoremediation of hydrocarbon-contaminated soils in the Sudd Wetland of South Sudan

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**Abstract**: Hydrocarbon contaminants have become a global concern due to their long-term adverse effects on soil ecosystems and human health. Successful implementation of phytoremediation to clean up hydrocarbon contaminants requires the identification of the most effective remediation plant species. Twelve native plant species of the Sudd Wetland in South Sudan were evaluated for their potential application as phytoremediators. The treatments included six total petroleum hydrocarbon (TPH) concentrations of 0, 25, 50, 75, 100 and 125 g/kg soil. The twelve native plant species tested were: *Sorghum arundinaceum* Desv., *Oryza longistaminata* A. Chev. & Roehrich, *Hyparrhenia rufa* Nees, *Abelmoschus ficulneus* L., *Gossypium barbadense* L., *Nicotiana tabacum* L., *Sorghum bicolour* L. Moench, *Eleusine coracana* Gaertn., *Capsicum frutescens* L., *Zea mays* L., *Tithonia diversifolia* Hemsl. and *Medicago sativa* L. Significant differences in phytoremediation rates were observed amongst the treatments with exception of the 125 g/kg soil concentration of hydrocarbon that was lethal to all the plant species. Over 50% TPH reduction in the 75 g/kg soil concentration was observed in contaminated soil phytoremediation in *H. rufa*, *G. barbadense*, *O. longistaminata*, *T. diversifolia* and *S. arundinaceum*, making them potential phytoremediators of hydrocarbon-contaminated soil in the Sudd-Wetland of South-Sudan.

**Keywords**: crude oil; soil contamination; toxicity; seed germination; Sudd native plants

Soil contamination with toxic petroleum products has received global attention due to their long-term adverse effects on soil ecosystems and human health (Newman 2009, Wang et al. 2017). Larger oil reserves are often coincidentally located in wetlands and river deltas (Wang et al. 2017), including the Sudd. The Sudd wetland in South Sudan is one of the world's largest wetlands covering approximately 5% (648 000 km$^2$) of the country (Ramsar Convention 2010) and has the largest crude oil reservoir (Rueskamp et al. 2014, Pragst et al. 2017). In the region, exploration and extraction of crude oil occur concurrently with agricultural activities (Tutdel 2010). As a result, remediation of petroleum hydrocarbon (PHC) is necessary.

Elsewhere, novel technologies such as soil excavation, solidification, deep burial, encapsulation and thermal stabilization methods have been used (Lim et al. 2016), but they are very expensive (Pal et al. 2016, Marinescu et al. 2017) and sometimes impossible on a large scale. Also, the soils remediated using these techniques are prone to secondary contaminations making the use of cost-effective alternative bioremediation-based technologies such as phytoremediation attractive (Masu et al. 2014, Marinescu et al. 2017). Phytoremediation involves the use of plants to clean up accumulated PHC in the soil (Njoku et al. 2016) as reported in numerous studies (Zand et al. 2016). These include grasses, vegetables, legumes, cultivated

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crops, ornamentals and some woody plants (Atangana et al. 2014, Shashsavari et al. 2016). To date, no phytoremediation research has been conducted in the Sudd region or other parts of South Sudan; yet, negative effects of crude oil extraction are already evident in the form of high concentration of salts in water, loss of livestock, reduction in vegetation cover and high content of heavy metals in the human body and diseases (Rueskamp et al. 2014, Pragst et al. 2017). Also, Mager et al. (2016) recently reported that over 500 active and abandoned oil and/or gas drilling sites require remediation. Also, Ruley et al. (2017) recently reported high concentrations of hydrocarbon contaminants in farmlands extending up to 5 km away from oil drilling sites in the Sudd region. These reports highlight the urgency for remedial treatment to rejuvenate soils of the Sudd region. For any successful implementation of phytoremediation trials, it is important first to identify the most effective remediation native plant species that are well adapted to the environmental conditions of a given region. The purpose of this study was to determine the capability of native plant species of the Sudd region, South Sudan to bio-degrade petroleum hydrocarbons from contaminated soil.

## MATERIAL AND METHODS

**Experimental layout**. Two pot experiments were conducted in a greenhouse during the period 2016 to 2017. The soil used was collected from non-contaminated natural land as composite topsoil samples at a depth of 0–30 cm from the Sudd region within latitudes 60°0'–90°30'N and longitude 300°10'–310°45'E at the elevation of 320 m a.s.l. (Ruley et al. 2017). The soil samples were air-dried and sieved to remove debris and a portion was taken for physico-chemical analyses in the laboratory (Table 1). No measurement of granulometric composition was undertaken since the soil used was extracted from areas that have not been subjected to the influence of the anthropogenic factor. All the other selected physicochemical properties were determined using the Standard Laboratory Method of soil analysis (Okalebo et al. 2002). The results are presented in Table 1. Samples of soil were artificially contaminated with known concentrations of crude oil. The treatments included five different concentrations of total petroleum hydrocarbon (TPH): 25, 50, 75, 100 and 125 g/kg soil, with 0 g/kg soil as control for plant biomass production. These were 29.3, 58.6, 87.9, 117.2 and 146.5 mL/kg soil of crude oil equivalent to total petroleum hydrocarbons of 25, 50, 75, 100 and 125 g/kg soil, respectively.

The crude oil was obtained from the Dar Petroleum Operating Company Ltd., Operation Base Camp in Paloch, South Sudan. Seeds of 11 plant species: *Sorghum arundinaceum* Desv., *Oryza longistaminata* A. Chev. & Roehrich, *Hyparrhenia rufa* Nees, *Abelmoschus ficulneus* L., *Gossypium barbadense* L. and *Nicotiana tabacum* L. commonly found in the oil-contaminated sites and *Sorghum bicolour* L. Moench, *Eleusine coracana* Gaertn., *Capsicum frutescens* L., *Zea mays* L. and *Tithonia diversifolia* Hemsl. commonly found in the Sudd region (Ruley et al. 2017) were collected from farm and natural sites; while seeds of *Medicago sativa* L. which had been used in several phytoremediation studies (Chekol and Vough 2001, Kaimi et al. 2006) were obtained from Sudan. No plant was used as a control (natural remediation) process of hydrocarbon-contaminated soil.

The treatments were laid out in a completely randomized design (CRD), with 78 treatments generated using the Genstat computer statistical software (Hertfordshire, UK), then replicated four times, making a total of 312 (6 hydrocarbon levels × 13 plant species × 4 replicates) treatment pots.

The soil was apportioned into 5 kg per pot for each treatment. To ensure that soil and the hydrocarbon were well mixed, each concentration of the hydrocarbon was thoroughly mixed with 5 kg soil on a metallic sheet and then returned into 8-L pots perforated at the base to facilitate drainage and aeration. Each pot was appropriately labelled to indicate the respective treatment and left for a week before planting.

Table 1. Physical and chemical characteristics of the soil used for the greenhouse experiment

| Parameter | Sand | Clay | Silt | STC | $pH_{H_2O}$ | P | N | SOC | CEC | K | Na | Mg | Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (%) | | | | | (mg/kg) | (%) | | (cmol$_+$/kg soil) | | | | |
| Test value | 24.234 | 61.292 | 14.474 | Clay | 6.71 | 15.64 | 0.27 | 5.01 | 13.84 | 1.69 | 0.94 | 1.25 | 9.92 |
| Critical value[a] | – | – | – | – | 5.5 | 15 | 0.2 | 3 | 10.9 | 0.5 | < 1.0 | 0.6 | 10 |

STC – soil texture class; SOC – soil organic carbon; CEC – cation-exchange capacity; [a]Critical value according to Okalebo et al. (2002) for most crops in East Africa

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The determination of seed viability was done through the floatation technique in which all seeds remaining at the bottom of the water were considered as potentially viable. Ten viable seeds of each plant species were sown in each pot, and the seedlings were thinned to three plants per pot. To minimize the generation of leachate, the pots were irrigated with deionized water up to field capacity at two-day intervals up to the end of the four-month test period. Leached water was collected using covers of the pots placed under each pot, and then re-used to irrigate the pots to minimize hydrocarbon loss. Periodically, the covers of the pots were rinsed with deionized distilled water, and the resulting wash solutions were poured back into the respective pots to minimize loss of hydrocarbon. Weeds emerging from some of the pots were hand-pulled. For the control of insect pests such as whiteflies, the foliar spray with dimethoate (0.05%) was done at an interval of 15 days.

**Data collection**. Data on plant seed germination, plant growth height, plant dry matter and percentage reduction of TPH were collected once, 120 days after planting. Following the measurements of plant height, the above-ground parts of the plant were cut off at the soil surface, followed by destruction of the pots for soil sampling. The compacted plants were shaken in a container to carefully collect the roots, washed under running tap water and air-dried to remove surface water. The plants were then partitioned into shoot and root systems. Fresh weights of the shoots and roots were taken. The partitioned plant parts were then oven-dried at 65°C to constant weight. The plant dry matter yield was obtained from the final weight of the oven-dried samples.

The soils collected after removal of the root parts from each pot, including the control, were first homogenized before being sampled for analysis of total petroleum hydrocarbon followed by storage at –4°C for further processing. 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 chromatography. TPH concentration was calculated according to the United States Environmental Protection Agency (USEPA) SW-846series, method 9071 B 5. The percentage of TPH degradation was calculated by subtracting the final gas chromatography results of the soil sample after harvesting of the specific treatments from the initial amount of TPH used at planting. The output was multiplied by 100%.

**Statistical analysis**. The data set for plant growth parameters (germination and plant height), yield (dry weight) and hydrocarbon remediation from the soil were subjected to the analysis of variance using the GenStat 17$^{th}$ Edition (VSN International Ltd., Hertfordshire, UK) to generate the treatment means by using the Fisher's *LSD* (least significant difference) test at 5% probability level.

## RESULTS AND DISCUSSION

**The response of plant growth parameters to different levels of TPH-contaminated soil**. The results demonstrated a significant reduction in all plant growth parameters (seed germination, plant height, and total plant dry weight) when compared to the control treatment. Seed germination rate was 100% for all the plant species in the control soil (i.e. soil with no TPH). Generally, a decrease in germination rate for all the plant species was observed with an increase in concentrations of TPH in the soil (Figure 1a). Germination of *H. rufa* and *O. longistaminata* was observed in crude oil contaminated-soil of 25–100 g TPHs, while the seeds for *E. coracana*, *C. frutescens*, *N. tabacum* and *A. ficulneus* germinated only in 25 g and 50 g of TPH in soil. The mean for the seed germination percentage in the TPH-contaminated soil differed among species, with the highest being observed for *H. rufa* (74.5%) and the lowest for *C. frutescens* (30%). In contrast, germination of alfalfa *(M. sativa)*, the phytoremediator control, was observed in contaminated soils up to 75 g TPH (Figure 1a). No germination occurred for either of the plant species in the 125 g/kg soil treatment. This could be due to the hydrophobicity of hydrocarbon-contaminated soils which prevented water infiltration and aeration required for proper growth of plant roots. The observation is supported by Odjegba and Sadiq (2002), Reichenauer and Germida (2008) and Vogelmann et al. (2013) who argued that where the TPH concentation is in extremes, aeration and mobilization of plant nutrients is impeded. In such a situation, it is eminent that plant germination, and consequently growth, is interfered with. Moreover, aerated and nutrient-rich soils present the potential factors for seed germination and plant growth to occur.

Four months after planting, all the plant species displayed a significant decrease in plant height when compared to the control (Figure 1b). The plant height response varied with the presence of different concentrations of TPH. The plants in the control treatment

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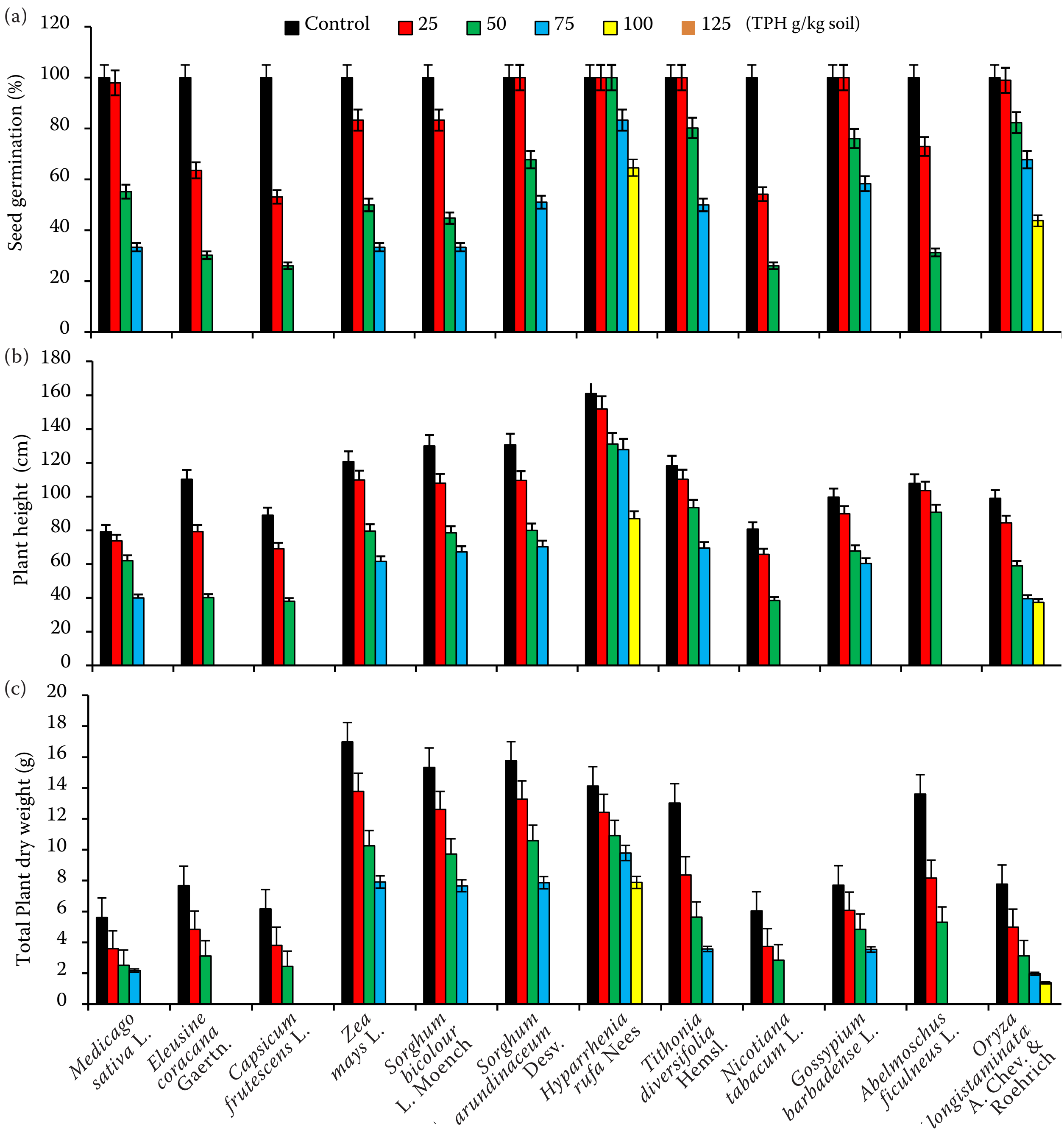


Figure 1. (a) Seed germination percentage under different concentrations of total petroleum hydrocarbon (TPH) in soil; effects of different concentrations of TPH soil contamination on (b) plant height and (c) total plant dry weight. Bars show the standard errors for percentage seed germination, plant height and total plant dry weight at $P < 0.05$, $n = 4$

grew taller than in the hydrocarbon-contaminated soil at 25, 50, and 75 g/kg, implying that TPH had an adverse effect. As earlier indicated, plant nutrients, air and water tremendously reduce with increase in the concentration of TPH in the soil. Deficiency in these conditions results in stunted plant growth. Similar results were reported in separate studies by Milala et al. (2015) and Umeh et al. (2017), who observed that presence of excessive hydrocarbons in a plant environment affects oxygen and nutrient transfer as it covers the roots and pores. It leads to asphyxiation of sub-surface roots especially in fine-textured (clay) or shallow soils with impervious subsoils. The small fibrous roots are deprived

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of oxygen leading to their death. In totality, this impairs the root system making it unable to supply the necessary water to replace that transpired by the leaves. This causes a water shortage leading to withering and/or death of plants.

Besides height, all plant species in the control treatment exhibited the greatest mean values for destructive pot plant biomass (Figure 1b). The below-and above-ground biomasses in terms of total plant dry weight were all significantly reduced with increasing concentrations 25, 50, 75 and 100 g of TPH when compared to the control of each plant species (Figure 1c). This testifies the findings of Wang et al. (2013) and Ribeiro et al. (2014) that the osmotic effects of crude oil create unfavourable conditions in the soil, consequently causing water stress and restricted availability of nutrients to plants. As a result, the germination of seeds and photosynthetic rates are interrupted, which reduces shoot height, stem density and overall biomass. The findings are also supported by Baruah et al. (2014) that plant biomass reduces with increased crude oil concentration, which they attributed to decrease in chlorophyll content. They further posit that the effect of chlorophyll reduction specifically results in plant shoot and root biomass and that shoot biomass is always higher than root biomass.

**Reduction of total petroleum hydrocarbon in contaminated soils**. Reduction of toxicity in TPH-contaminated soils was experimented through phytoremediation under greenhouse conditions using various Sudd wetland plant species. The total removal rates of TPH were determined, and significant differences in the removal rates were observed among all the plant species (Table 2). After 120 days of phytoremediation, the mean percentage removal rate of TPH ranged from 12.7% to 48.54% in the contaminated soils, higher than that in control by 11.41% (Table 2). Four plant species, *C. frutescens* (12.7%), *E. coracana* (13.34%), *N. tabacum* (13.49%) and *A. ficulneus* (14.19%), had the lowest mean percentage removal rate; compared to that, the mean percentage removal rate of *H. rufa* was the highest (48.54%). More than 30% mean percentage removal rates were observed in *O. longistaminata* (36.25%), *G. barbadense* (34.86%) and *T. diversifolia* (33.32%) (Table 2).

When considering the concentration of TPH-contaminated soil at 75 g/kg at which most plants germinated, over 50% reduction of TPH was observed in *H. rufa* (74.4%), *G. barbadense* (66.27%), *O. longistaminata* (56.17%), *T. diversifolia* (55.92%) and *S. arundinaceum* (50.12%). Moreover, *H. rufa* and *O. longistaminata* showed promising phytoremediation efficiency in 100 g hydrocarbon-contaminated soils, with 60.7% and 52.56% reductions potential, respectively. TPH remediation decreased in all the plant species at 125 g/kg (Table 2) because of the high

Table 2. Hydrocarbon degradability potential of native plants in Sudd region

| Plant species | The concentration of total petroleum hydrocarbon (g/kg soil) | | | | | Mean | Mean ranking |
|---|---|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | 125 | | |
| *Medicago sativa* L. | 39.60 | 43.18 | 49.60 | 9.09 | 10.32 | 25.29 | 6 |
| Control | 15.20 | 20.96 | 14.80 | 8.30 | 9.61 | 11.41 | 13 |
| *Eleusine coracana* Gaertn. | 21.20 | 27.40 | 15.48 | 8.50 | 9.87 | 13.34 | 11 |
| *Capsicum frutescens* L. | 18.10 | 23.60 | 15.73 | 8.20 | 10.56 | 12.7 | 12 |
| *Zea mays* L. | 28.35 | 36.85 | 42.00 | 8.90 | 10.16 | 21.04 | 7 |
| *Sorghum bicolour* L. Moench | 24.35 | 32.60 | 38.40 | 9.00 | 10.56 | 19.15 | 8 |
| *Sorghum arundinaceum* Desv. | 42.50 | 44.83 | 50.12 | 9.78 | 10.96 | 26.36 | 5 |
| *Hyparrhenia rufa* Nees | 70.00 | 74.00 | 74.40 | 60.70 | 12.16 | 48.54 | 1 |
| *Tithonia diversifolia* Hemsl. | 47.95 | 50.35 | 55.92 | 10.33 | 10.36 | 33.32 | 4 |
| *Nicotiana tabacum* L. | 18.40 | 25.80 | 17.33 | 9.76 | 9.60 | 13.49 | 10 |
| *Gossypium barbadense* L. | 59.65 | 59.80 | 66.27 | 12.16 | 11.30 | 34.86 | 3 |
| *Abelmoschus ficulneus* L. | 20.00 | 26.08 | 17.20 | 12.10 | 9.76 | 14.19 | 9 |
| *Oryza longistaminata* A. Chev. & Roehrich | 47.65 | 50.02 | 56.17 | 52.56 | 11.12 | 36.25 | 2 |
| SEM = 0.73; $LSD_{0.05}$ = 2.03; *CV* (%) = 8.1 | | | | | | | |

SEM – standard error of the mean; *LSD* – least significant difference; *CV* – coefficient of variation concentration of petroleum hydrocarbon. *T. diversifolia* (a legume) and *H. rufa* (grass species) showed high potential of TPH remediation among all the plant species. This finding rhymes the observations made in numerous studies in which it is reported that the grasses (Poaceae) and legumes (Leguminosae) are effective in the clean-up of contaminants from the environment (Merkl et al. 2005, Zand et al. 2016). More light about the suitability of legumes and plant species is shed by Merkl et al. (2004) and Zand et al. (2016) who conclude that grasses and legumes are the best candidates for phytoremediation process owing to their multiple and larger root surface area. Further, they espouse that legumes can fix atmospheric nitrogen by potentially replenishing what is lost from the soil during oil spills.

Various studies on phytoremediation have reported that alfalfa is the best phytoremediator. While this study has also proved the same, beyond, it has proved that *H. rufa* has more potential than alfalfa. This study is the first of the kind to unravel this potential among Sudd native plants. Secondly, this study also came up with a novel and unique finding regarding the phytoremediation potential of leguminous plants. It was proved that in the Sudd region, *T. diversifolia* has greater potential for phytoremediation than alfalfa (*M. sativa*) although different studies have ranked alfalfa as the best. Also, this study brings new knowledge to the fore regarding the ability of the plant species *H. rufa*, *G. barbadense*, *O. longistaminata*, *T. diversifolia* and *S. arundinaceum* to thrive in hydrocarbon-contaminated soils as it was also proved that such soils have less pronounced effects on their growth parameters.

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