

## **Phytoremediation Treatment of drilling cuts contaminated with petroleum hydrocarbons using Bermuda grass (Cynodon dactylon)**

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### **Abstract**

Phytoremediation of drilling waste contaminated soils was tested using a native Bermuda grass species. A glasshouse experiment evaluated the ability of grass to survive, degradation of petroleum hydrocarbons in contaminated soils. In this study, Bermuda grass was planted in soil comprising different ratios of soil: waste to examine the effect of petroleum hydrocarbons concentrations. Biomass measurements including shoot biomass, grass height, leaf area, roots height and density were made, in addition to testing for any TPHs contamination, and the role of microorganisms and enzymes in the dissipation of petroleum hydrocarbons. This research suggested that Bermuda grass is a useful species for phytoremediation of soils contaminated by drilling waste. Bermuda grass also is shown to have the potential to remediate soils contaminated by petroleum hydrocarbons when the contamination level below 7350 mg kg<sup>-1</sup> TOC. The grass offers an environmentally-friendly, cost-effective waste management option for some sites despite requiring a longer time. Results from this study are helpful for further field phytoremediation studies.

**Keywords:** Phytoremediation, Bermuda grass, TPHs contamination Biomass.

### **1. Introduction**

The process of drilling oil and gas wells for exploration and production generate massive quantities of drilling waste contains cutting and spent drilling fluids. This type of waste classified as 'toxic waste' cause environmental problems and considered one of the main problems facing the oil and gas industry in Libya due to the significant negative ecological impacts to the surrounding environment. Up to now, in most of drilling operations in Libya, drilling waste temporarily stored in earthen pits close to drilling operations before disposal to the land or buried in theses pits. This waste management option provides a simple and low cost disposal method, but its unsuitable solution for wastes that contain high concentrations of organic materials and heavy metals and other harmful components that could migrate from the pit to cause pollution of surrounding water bodies, soil and air (Biltayib .2006). Engineering techniques used in treatment of soil contamination based on physical, chemical and thermal processes are very expensive and not always effective. Biological treatment techniques are perhaps the most suitable in Libya due to their low-inputs and low- cost. Further work is required to specifically assess the efficiency of these approaches for the remediation of drilling waste in the context of the Libyan environment. The biological treatment solutions such as bioremediation has become a valuable alternative to chemical and physical (traditional) methods.

Bioremediation has low operation cost (costs 10-20% of other mechanical treatments) (El-Dars et al. 2016), and can be used efficiently in removing of organic and heavy metals from contaminated water and soils. Bioremediation treatment techniques divided into two broad categories in-situ and ex-situ media. In-situ treatment processes involve treatment of contaminated soils and water in the place of generation, without removal to different site for treatments which reduce the cost of treatment, where in Ex-situ treatment processes, the contaminated media transferred to a treatment area (US EPA. (2018)), among the bioremediation techniques, Phytoremediation has emerged as good choice to detoxify polluted sites (Soils, water and sediments) in-situ. Phytoremediation is the use of vegetation to minimise the contamination volume, mobility of contaminants, or toxicity of contaminants in soil, groundwater, or other contaminated media (USEPA, 2000; Etim 2012). It is defined as a biological technology use plants for in situ removal, degradation, or clean-up of different types of pollution including metals, pesticides, explosives, and oi contaminants in different contaminated media (soils, sludge, sediments, surface water and groundwater), the used plants also help stabilise contaminates and reduce its mobility from sites by wind, rain, and groundwater to other areas. The most important factors affecting the successful phytoremediation are plant type and addition of soil amendments and bulking agents (ElDars et al. 2016). Phytoremediation is best implemented at sites with lower contamination by organic materials or heavy metals and usually applied through one of these mechanisms: Phytotransformation, Rhizosphere Bioremediation, Phytostabalisation, Phytoextraction, Phytovolotalisation or Rhizofiltration. Among the various mechanisms involved in phytoremediation of contaminated soils, Rhizodegradation is of major significance for the enhanced removal of hydrocarbons from soil (Lijuan Cheng et al. 2019). The performance of Rhizodegradation mechanism enhanced by root exudates such as enzymes and flavonoids, organic acids, sugars and amino acids which play the major role to induce bacteria and fungi growth in rhizosphere soil and consequently leading to degradation and mineralization of hydrocarbons pollutants (Dadrasnia, and Ismail, (2015)). Several plants already identified and trailed to be used in the rizeoremidation of organic compounds and heavy metals (Anyasi and Atagana. (2018)), among of them wild grass species such as Bermuda grass (*Cynodon dactylon*) and Tall fescue (*Festuca arundinacea* Schreb) and stargrass (*Ponterderiaceae*). The selection of Bermuda grass because it is grows well on a variety of soil from heavy clay to deep sands, it tolerates both acid and alkaline soil conductions and is highly tolerant to salt, drought, anoxia (flooding), cold and soil compaction (Buble 2009). Moreover, it has extensive, fibrous, widely branched and deep root systems which can provide significantly larger root surface area for colonization by microorganism in its rhizophere (Turgeon 1980) are mainly restricted by hydrophobic and toxicological nature of the contaminants, as well as the synergy existing between plant roots and soil microorganisms (Wang et al. 2011). In most of previous studies, the phytoremediation of hydrocarbons using Bermuda grass was conducted in natural soil or synthetic soil, there is no available studies

conducted in drilling waste cutting or soils with calcareous nature which may affect the growth of the grass and effect all of biodegradation process. According to the principles of phytoremediation treatment of hydrocarbons through Rhizodegradation mechanism, the main objective of this research is to test the tolerance levels of Bermuda grass species to grow in drilling waste contain high concentration of petroleum hydrocarbons and have a calcareous nature and the adoption of this species to the desert climate in Libya, and providing a preliminary evaluation of the effectiveness of Bermuda grass as potential phytoremediater species to clean up and/or reduce the contamination in drilling waste .

## **2. Material and Methods**

### **2.1 Site and Soil samples collection**

Two different drilling waste samples and uncontaminated native soil sample for experiment were collected during the drilling operations in Jalu area in Libyan Desert. The physical properties, chemical composition and texture of soil samples shown in table 1. All samples were dried in air for 2 weeks, sieved through 2 mm mesh to eliminate coarse rock and plant material, and thoroughly mixed to ensure uniformity.

Table 1. Physical properties, chemical composition and texture of soil samples

Test	Unit	WBMW	OBMW	N. Soil
pH	-	8.4	8.8	8.7
EC	ms/cm	4.06	4.2	0.08
T.D.S	mg/Kg	2035	1995	38
Moisture	Wt. %	12.4	55.2	3.79
L.O.I	Wt. %	21.3	59.5	4.35
TPH	Wt. %	1.47	8.56	0.15
Cr	mg/Kg	28.4	34.3	10.8
Mn	mg/Kg	43.3	33.7	80.1
Cu	mg/Kg	9.3	34.5	16.4
Zn	mg/Kg	50.9	74.8	19.3
As	mg/Kg	2.0	6.17	1.37
Cd	mg/Kg	0.44	0.72	0.0
Ba	mg/Kg	1066	3832	242
Pb	mg/Kg	10.3	61.0	7.07
Na	mg/Kg	2089	2217	1328
Ca	mg/Kg	280972	253821	5978
Mg	mg/Kg	2211	19523	1843
K	mg/Kg	1279	11306	7218
Cl	mg/Kg	8400	6590	112
NO3-N	mg/Kg	0	0	0
PO4-P	mg/Kg	0	0	0
SO4-2-S	mg/Kg	303	2152	120
Mean Particle Size	□m	314	193	124

Specific Surface Area	cm <sup>2</sup> /g	2043	3173	795
Soil Texture	-	Loamy Sand	Loamy Sand	Sand
Minarology by XRD		Calcite CaCO <sub>3</sub> Quartz SiO <sub>2</sub> Ankerite Ca(Fe <sup>2+</sup> ,Mg)(CO <sub>3</sub> ) <sub>2</sub>	Calcite CaCO <sub>3</sub> Quartz SiO <sub>2</sub> Bairite BaSO <sub>4</sub>	Quartz

WBMW= Water based mud waste    OBMW= Oil Based mud waste    N. Soil= Native Soil

### • Bermuda Grass

Commercially Bermuda grass (*Cynodon dactylon*) was selected in these experiments due to its availability in Libya and the gained experience in adapting of this plant to the harsh conditions, such as heat, extensive light, and drought.

## 2.2 Green House Experiments

Pot experiments were conducted during the summer period (June, July and August) in order to grow the candidate grass for 2 months (60 days) in contaminated soil at greenhouse condition similar as possible to Libyan Desert climate. The drilling waste and native soil samples were supplied with a compound fertilizer (N : P<sub>2</sub>O<sub>5</sub> : K<sub>2</sub>O = 10: 8: 9) to compensate the shortage of essential nutrients (N & P) in drilling waste and native soil samples and kept for equilibration for 1 week in air then sieved through a 2 mm mesh sieve.

### 2.2.1 Pot experiment set-up

The experimental design contains three different treatments (Control, WBMW and OBMW). In control treatment only native soil was used where in WBMW and OBMW treatments, different TPH and HMs contamination levels were prepared by dilution of contaminants in drilling waste samples using native soil. Table 2 illustrates the treatments and approximation concentration of TOC in each treatment.

Table 2. Description of pot experiment design

Code	Treatment	Dilution Factor	TOC Level Wt. %	Approx. TOC concentration mg/Kg
C	Control (Native Soil)	1	-	-
25W	25WBMW:75Native Soil	4	0.37	3675
50W	50WBMW:50Native Soil	2	0.74	7350
100W	100 WBMW	1	1.47	14700
25O	25OBMW:75Native Soil	4	2.14	21400
50O	50OBMW:50Native Soil	2	4.28	42800
100O	100 OBMW	1	8.56	85600

Each treatment was replicated 4 times, making a total of 28 pots. About 300 g of soil samples (Control or contaminated soil) were added to a plastic pot with a radius of 7.0 cm and a height of 15 cm. According to the results obtained from germination tests of Bermuda grass in native soil, seeding of 50 g /m<sup>2</sup> selected, and similar seeds

weight sow in each replicate. All pots arranged randomly and exposed to greenhouse condition for 60 days at temperature controlled between 25 to 35 °C and humidity between 40- 60%.

The watering for all pots were adjusted to 30% of water holding capacity of each treatment (0.1 ml/g) to avoid leaching of hydrocarbons and heavy metals from soil. In addition, a 10/1000 strength liquid fertilizer (N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O = 10: 8: 9) was applied weekly. After 60 days, the plants extracted carefully from each pot then divided into roots and shoots by cutting the aboveground parts of the plants at the soil surface. Both parts sealed in plastic envelopes **Analysis** :For measurement of biomass, the root parts of grass were washed thoroughly with tap water then by deionised water to remove soil particles, then placed in paper bags and dried in oven at 45 °C for 72 hours, the average of roots length and dry weight was measured. The physical analysis of the soil samples that was firmly attached to the roots conducted using conventional methods. The chemical analysis was conducted using Gas Chromatography equipped with flame ionisation detector ( GC-FID) type Agilent model 7890 for measurement of total petroleum hydrocarbon (TPH) concentration. The microbial activity in rhizosphere soil was evaluated using Biolog plate and the microbial enzymes activity was evaluated by measuring the (DHA)

### **3. Results and dissection**

**Drilling waste samples analysis:** The physical, chemical and texture analysis of the drilling waste samples prior to grass planting as given in Table 1 shows that both samples have sandy loam texture, classified as alkaline soil and considered as calcareous soil have high content calcium carbonate. The concentration of calcium in both drilling waste cuts ( WBMW & OBMW) very high 280972 and 253821 mg/kg respectively which detrimental to turf grass, causing damage to grass leaves and blades and affect growth of candidate grass. The analysis results indicate that both drilling waste cuts classified as heavy petroleum-contaminated soils containing high concentration of petroleum hydrocarbons, low levels of toxic heavy metals such as copper, manganese and chromium, and very poor nutrients (Phosphors & Nitrogen) which expected to have negative impact in grass growth.

#### **Grass Growth and Biomass measurements**

Bermuda grass showed a promising behaviour and high potential of adaptation to soils contaminated by low levels of petroleum hydrocarbons ( after dultion of raw WBMW&OBMW using native soil) as shown by the growth and biomass production in control, 25W and 50W treatment during a period of 60 days in greenhouse condition. There are no visible adverse symptoms such as wilting, lodging or defoliation observed before harvest during the study period. The response of Bermuda grass to TPH and HMs contamination level was ranging from reasonable growth in control and 25W treatment to moderate effect in 50W treatment. The grass growth in treatments 100W, 25O, 50O and 100O were severely stunted, thus this treatment will not be considered in the further discussion in plant development or phytoremediation of petroleum hydrocarbons. The results showed that no grass growth in all treatments contains more than 7350 mg/kg TPH which may attribute to the high levels of petroleum hydrocarbons and the oily nature of this treatments.



Figure 1. Reduction in plant parts length in different amended WBMW treatments

As shown in figure 1, Bermuda grass exhibited good growth rates in native soil (control

0.0 % wt. %. Approx. 0 mg/Kg TOC) and in WBMW diluted treatments, 25W (0.37 % wt. Approx. 3675 mg/Kg TOC) and 50W (0.74 % wt. % 7350 mg/Kg TOC). The rate and extent of grass growth in control and 25W was almost the same where it is reduced by approx. 16 % in 50W treatment. The plant height decreased significantly with increasing in the contamination level ( $p < 0.001$ ). No difference in average grass length between control and 25W, in both treatment was almost similar ( approx. 29 cm) after 60 days. However, plant Length reduced by approx. 16% with increasing of the contamination level in treatment 50W and by approx. 86 % in treatment 100W when compared with the control as shown in figure 2.

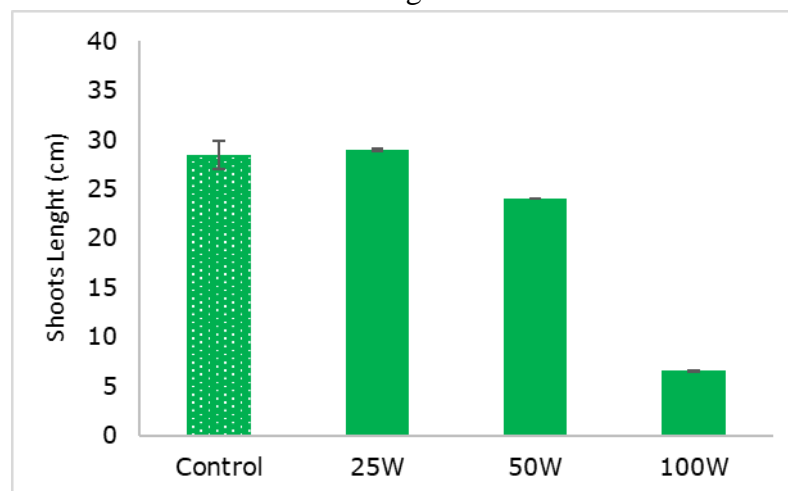


Figure 2. Plant height of Bermuda grass in different WBMW treatment

The results showed that roots density and length different qualitatively and quantitatively between the treatments. The roots length in successful treatments decreased significantly ( $P=0.001$ ) as the contamination level by HMs and TPH increased. The root of plant in



control and 25W treatment were distinctly larger and more fibrous than the root system in 50W treatment. The root length reached the highest level in treatment 25W, and then declined. Besides, no significant ( $p > 0.1$ ) decrease was found in root length and root weight between control and 25W treatments as shown in table 3 and figure 1 .

Table 3. The changes in plant parts length under different contamination levels

Treatment	Control	25W	50W	100 W
Length of plant parts				
Shoots (cm)	28.5	28.9	24.0	4.1
Reduction (%)		+ 1.4	-16	-86
Roots (cm)	7.8	8.0	5.7	0.5
Reduction (%)		+ 2.5	-22	-93

### Biomass and leaf area Measurements

Based on the results illustrated in figure 3 of biomass measurements (Dry weight), it was found that the total biomass yield per pot decreased significantly as the contamination level increased. The biomass measurements of treatment 25W did not reduced significantly when compared with the control, but contrary to the expectations showed reasonable outgrowth (approx. + 40%) in dry shoot weight, whereas that a significant ( $p < 0.001$ ) reduction was observed in treatment 50W and 100W (-48 % and -97% ) respectively as shown in figure 4.

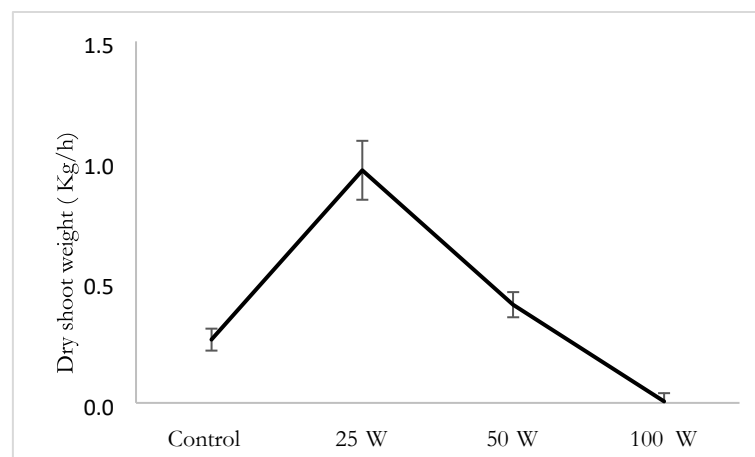


Figure 3. Total biomass (dry weight) of Bermuda grass in different WBMW treatment. .

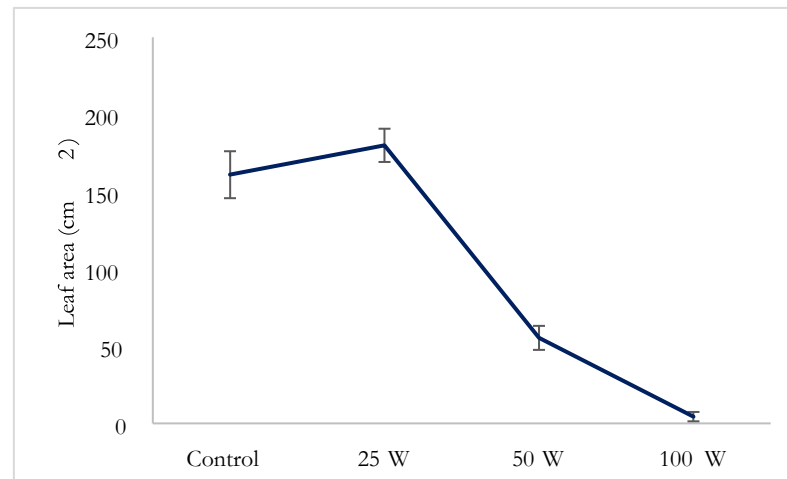


Figure 4. Leaf area of Bermuda grass in different WBMW treatment.

The obtained results support the finding of many researchers whom concluded the destructive effect of high levels of petroleum hydrocarbons and heavy metals in grass growth (Basumatary et al. 2012 ; Htwe et al. 2016). Overall, petroleum hydrocarbons found to have an inhibiting plant growth and effect the general plant health (especially root system), duo to the toxicity nature of these types of compounds. This finding broadly supports the work of other studies in this area linking the survival of different grass species in soils contaminated by petroleum hydrocarbons. Different authors observed similar adverse effect on plant growth in soils contaminated by hydrocarbon. (Razmjoo et al. , 2012; Saraeian et al. 2016) was reported reduction in shoot and roots growth of Bermuda grass planted in soil contain 6 wt. % petroleum hydrocarbon contaminates. Bermuda grass shoot fresh and dry weight decreased significantly with increasing in the level of contamination.

#### **Effect of Soil texture and changes in soil properties .**

During the watering procedure for the plants in the greenhouse experiments, it noticed that the treatments 100W, 25O, 50O and 100O have very low water holding capacity and showed low permeability and low infiltration for irrigation water and looked as flooded soil. Contrary, the other treatments such as control, 25W and 50W exhibited good water holding capacity and reasonable grass growth. Also, it noticed that the treatments which amended by addition of native soil for the purpose of dilution of contamination level gave better grass growth comparing with the un-amended treatments which may attributed to different reasons as mentioned in literature:

- i) Native soil played the role of bulking agent and increased the soil porosity and the aeration in 25W and 50W treatment, thus, increased the oxygen diffusion and the microbial activity needed for grass growth (El-Dars et al. 2016)
- ii) The hydrophobic nature of treatments 25O, 50O 100W and 100O as a result of high TPH content led to low permeability and low infiltration of water in these treatments causing artificial drought in the subsurface layer of soil in those treatment which affected negatively on grass growth (Edama et al. 2011).



iii) The higher clay content in the unsuccessful treatments leads to binding of the soil particles by the effect of irrigation water which governing water, air, bioavailability of nutrients and temperature in soil, which in turn, govern plant growth.

The changes in pH value of soil After 60 day greenhouse experiment illustrated in table 4. The obtained results shows that pH value of the control, 25W and 50W treatments exhibited good grass growth was in the range of 7.3, which is close to the optimum pH value recommended by Pawar, R. (2015) to enhance the bioavailability of essential nutrient needed for grass growth (pH 6.0 -6.5), and very close to the pH value needed for Phytoextraction of heavy metals as recommended by Htwe et al. (2016).

Table 4. Changes of pH-value after planting of Bermuda grass in different treatments

<b>Treatment</b>	<b>control</b>	<b>25W</b>	<b>50W</b>	<b>100W</b>	<b>25O</b>	<b>50O</b>	<b>100O</b>
<b>Before Planting</b>	8.7	8.4	8.4	8.4	8.4	8.8	8.8
<b>After planting</b>	7.2	7.3	7.3	10.8	11.1	11.3	11.6

In the unsuccessful treatment such as 100W, 25O, 50O and 100O, it is apparent that the high pH values result from the high calcium carbonate content in these treatments which lead to less vigorous growth and nutrient deficiencies and inhibiting the Bermuda grass growing in these treatments.

### **Effect of TPH on phytoremediation by Bermuda grass**

The biomass measurements illustrated in Figures (1,2,3 and 4) in this research showed that the shoot growth of Bermuda grass reduced scientifically (p-value <0.001) as the petroleum contamination levels in the soil increased. The same effect (p-value < 0.001) was recorded in roots density and leaf area as petroleum contamination levels increased up to 0.74 wt.% TOC (approx. 7350 mg kg<sup>-1</sup>).

Despite the fact that hydrocarbon pollution depressed plant growth to some extent as the petroleum hydrocarbon concentration increase, the results indicate that the Bermuda grass can survive and tolerate petroleum hydrocarbons concentration below (0.74 wt. % approx. 7350 mg kg<sup>-1</sup> TOC), and it is can efficiently dissipate the petroleum hydrocarbon from amended drilling waste, indicating that phytoremediation, using this species is useful technique for remediation of soils contaminated by drilling waste polluted with petroleum hydrocarbons in level < 0.74 wt.% TOC. Similar results were obtained by Yateem et al., (2000) using alfalfa and perennial ryegrass as phytoremediater plant in soil contaminated by the presence of 1% TPHs. The chemical analysis of the rhizosphere soil for treatments 25-W and 50-W demonstrated 99.0 and 98.0 % reduction in TPH content respectively (table 6), compared with 32% reduction in the nonplanted controls after the bioremediation treatment for 60 days. The results indicate that the higher reduction in TPH content was in the rhizosphere region (almost 67 % in both treatments). The reduction of

TPH in unplanted control may be attributed to the evaporation by the action of glasshouse temperature changes (25-35°C) during the glasshouse experiment period, or to the microorganism activity in this samples.

Table 5: TPH concentration in leachate of planted soil contaminated by drilling waste in treatments (25-W and 50-W) after glasshouse experiment for 60 days

TPH Fraction	TPH (mg kg <sup>-1</sup> ) in leachate of planted treatments	
	25-W	50-W
n-C10	0	0
n-C11	0.00	0.00
n-C12	0.00	0.00
n-C13	0.00	0.00
n-C14	0.00	0.00
n-C15	0.00	0.00
n-C16	0.00	0.00
n-C17	0.66	1.32
n-C18	0.00	0.00
n-C19	0.00	0.00
n-C20	0.43	0.87
n-C21	0.69	1.37
n-C22	0.00	0.00
n-C23	1.40	2.81
n-C24	0.06	0.11
n-C25	0.00	0.00
n-C26	0.00	0.00
n-C27	0.00	0.00
n-C28	0.00	0.00
n-C29	0.00	0.00
n-C30	0.00	0.00
n-C31	0.00	0.00
n-C32	0.00	0.00
n-C33	0.00	0.00
n-C34	0.00	0.00
<b>Total TPH conc</b>	3.24	6.48

The efficiency of Bermuda grass in removal of petroleum hydrocarbons from amended drilling waste samples was expected when compared to previous studies using Bermuda grass in remediation of petroleum hydrocarbons contaminated soil. In accordance with the present results, Kaimi et al., (2007) obtain similar results, by comparing the efficiency of Bermuda grass with another twelve-plant species in

removal of TPH from soil contaminated by (1.5 wt.% TOC) and they found more than 65 % reduction in TPH caused by the plant and almost 37% reduction was lost by evaporation. The results also in line with Saraeian et al., (2016) who found that Bermuda grass reduce the petroleum hydrocarbon content to approx. 40% from soil contaminated by 2 % wt. TOC, and with Razmjoo et al., (2012) where degradation was reported to be 41 % from soils contaminated by 6 % wt. TOC. This study also confirmed these findings and furthermore showed that Bermuda grass tolerate contamination by petroleum hydrocarbons levels below (0.74 wt. % TOC) and can maintain its growth when faced different stress applied in this experiment such as waste texture, calcareous nature of the waste, contamination by TPH and HMs and Desert climate changes.

### **Effect of plants on soil microbiology**

The obtained Biolog Plate results used as a qualitative marker to evaluate the diversity and population of microorganisms able to survive in in drilling waste and metabolize the petroleum hydrocarbons. Soil microbiology activity were investigated using BIOLOG-GN2 plates in order to obtain an idea about the variation of microbial diversity in the planted drilling waste samples. The obtained results also used to know how this variation is influenced by the different environmental stresses such as the high TPH concentration, the calcareous nature of drilling waste and the desert climate changes. The results at the end glasshouse study showed that the bacterial enumeration were higher by orders of magnitude in the amended drilling waste planted treatments. The obtained data also confirmed that the growth of the Bermuda grass in amended drilling waste significantly augmented the number of biodegradative bacteria in the root zone for treatments 25W and 50W as well as the activity and diversity of the microbial communities. The general trend was an increase of the proportion of biodegrading bacteria in the microbial community during the treatment in the rhizosphere soil in all planted soil samples. The obtained data corresponds with findings made in similar studies that have assessed microbial counts in contaminated soils (Mcintosh et al. 2015; Varjani and Upasani, 2017).

These experiments confirm that the addition of plants and soil amendments increases bacterial counts compared to unplanted soils and also confirms that Bermuda grass could tolerate the contamination levels in amended drilling waste, and that the rhizosphere soil in planted samples contains survived organisms can tolerate the stress such as TPH contamination, heat and the calcareous nature of drilling waste, and that Bermuda grass will be effective in terms of bioremediation of petroleum hydrocarbons through a rhizosphere effect.

### **Soil enzymes activity assays**

In order to assure the correct sequence of biochemical reactions in the planted treatments, the soil dehydrogenase enzymes activity DHA was measured after 60 days

glasshouse experiment, and used as indicator of soil quality changes resulting from phytoremediation management practices using Bermuda grass. The dehydrogenases enzymes representing the most important enzymes in rhizosphere soil and belonging to the ‘oxidoreductases’ class and being used as indicator in the soils microbial activity during the phytoremediation of contaminated soils (Quilchano and Marañón, 2002) (Salazar et al., 2011). The results in figure show the dramatically increase in DHA activity in treatments 25W and 50W after cultivated by Bermuda grass for 60days and a little change in the control samples.

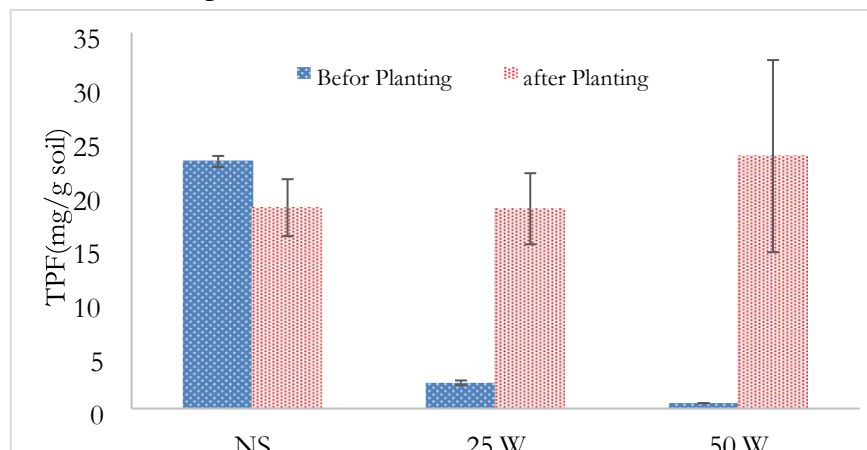


Figure 5. Activity levels of soil dehydrogenases enzymes in remediated soil

The obtained results showed enhancement of DHA values in planted treatments, indicating that the metabolism of soil microbes was promoted, thus resulting in the enhanced microbial which indicate that the general activities of microbes in planted soil were enhanced (Teng et al. 2015; Alrumman et al. 2015). Treatment 50W showed the highest increase in DHA value where treatment 25W and the control shows similar DHA values. From the DHA activity data, the stimulation of petroleum hydrocarbon degrading microbes expected in all successful treatments and that the enzymatic dehydrogenation effect of plants on organic compounds was greatly enhanced under low-level hydrocarbon contamination stress; thereby expected to be effectively promoting and improving degradation of TPHs in rhizosphere soil.

## Conclusion

Throughout this study, several evidence was presented that Bermuda grass is an important plant species that can be effectively applied as phytoremediator for clean-up of heavy metals and petroleum hydrocarbon from drilling waste and can efficiently survive and tolerate petroleum hydrocarbons concentration below (0.74 wt. % □ 7350 mg/Kg)

- Phytoremediation of petroleum hydrocarbons using Bermuda grass has proven to be a feasible method for remediation of drilling waste contains heavy metals and petroleum hydrocarbons less than 0.74 wt.% in the Libyan desert climate.
- It was concluded that the degradation of petroleum hydrocarbons was enhanced through a rhizosphere effect by the effect microorganisms, which through its diversity, density and activity degrade hydrocarbons and facilitate the mobility of heavy metals in the polluted soil.
- The presence of high concentrations of petroleum hydrocarbons reduce the biomass and relative growth rate as the concentration increase.
- Amendment of drilling waste by addition of native soil have larger particle size increase the soil porosity, aeration, oxygen diffusion and water holding capacity in contaminated soil, thus enhance the microbial activity which reflects positively on the phytoremediation efficiency of candidate grass.

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