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Phyto-remediation of Petroleum Hydrocarbons in Drilling Tailings, Libya

Aisha S. M. Amer

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Author affiliation
Department of Biology, Faculty of Education (Gasar Bin Ghashir), University of Tripoli, Libya
.
amer.aisha@yahoo.com
Ai.Amer@uot.edu.ly
Tel: 0943764311

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ABSTRACT

Phytoremediation of drilling waste, and contaminated soils were tested using a commercial mixture of poaceae, (*Festuca arundinacea*, *Poa pratensis* and *Lolium perenne*). The experiment evaluated the ability of grass to survive, and the degradation of petroleum hydrocarbons in contaminated soils. The mixture of grass was planted in soil comprising different ratios of soil: waste (0, 25, 50 and 75% of water-based drilling waste) to examine the effect of a range of total petroleum TPHs concentrations. Biomass measurements including shoot and root biomass, grass height, and leaf chlorophyll content, were made, in addition to determining the occurrence of any reduction in leachability of total petroleum hydrocarbons contamination. Finally, the role of microorganisms and enzymes in the dissipation of petroleum hydrocarbons was observed. This research found that the mixture is useful for the phytoremediation of soils contaminated by drilling waste. The grasses also are shown to have the potential to remediate soils contaminated by petroleum hydrocarbons when the contamination level is below 50% (0.25 wt. % or 2488 $mgKg^{-1}$ TOC) where the petroleum hydrocarbon concentration in each successful treatment (25% and 50%) were removed by the efficiency of gross petroleum hydrocarbon degradation of 74 %, and 59.6 %, respectively. The findings also prove that the use of grasses mixture enhances the microbial enumeration in the root zone which is expected in helping the uptake and/or degradation of petroleum hydrocarbons for up to 52 days. The mixture of grasses offers an environmentally friendly, cost-effective, waste management option for some sites despite the need for long time. These findings indicate that more studies are much needed in the field of phytoremediation.

المعالجة النباتية للهيدروكربونات البترولية في مخلفات الحفر في ليبيا

عائشة صالح إمحمد عامر

المعالجة النباتية باستخدام بعض أنواع النجيليات الفستوكة *Festuca arundinacea*، قبأ المروج *Poa pratensis* ، الزوان *Lolium perenne* تم تقييمها كأحد أهم الخيارات المستخدمة في معالجة الحمأة النفطية الناتجة من عمليات إنتاج النفط والغاز وذلك لأنه غالبًا ما يتم إعاقة المعالجة الميكروبية لهذه النفايات بسبب سميتها العالية. تم اختبار المعالجة النباتية لتربة ملوثة بمخلفات الحفر باستخدام خليط تجاري من أنواع حشائش. قيمت هذه التجربة قدرة الحشائش على البقاء و تحليل او تبديد الهيدروكربونات البترولية في التربة الملوثة، حيث تم زرع خليط الحشائش في تربة تحتوي على نسب مختلفة من النفايات (0 ، 25 ، 50 ، 75٪ من نفايات الحفر المائية). لفحص تأثير تركيزات مختلفة من الهيدروكربونات البترولية تم إجراء قياسات كل من الكتلة الحيوية للمجموع الخضري و الجذري وارتفاع العشب، ومحتوى الأوراق من الكلوروفيل و دور الكائنات الحية الدقيقة والإنزيمات في تفكيك وتبديد الهيدروكربونات البترولية بالإضافة إلى تحديد حدوث أي انخفاض في تركيز الكلي للهيدروكربونات البترولية. أثبتت النتائج أن الخليط العشبي مفيد لمعالجة مخلفات الحفر الملوثة للتربة حيث تبين أن الأعشاب لديها القدرة على معالجة التربة الملوثة بالهيدروكربونات البترولية عندما يكون مستوى التلوث أقل من 50٪ (0.25 ٪ بالوزن أو 2488 مجم/كجم

من الكربون العضوي الكلي) حيث تم إزالة هذه الملوثات في كل معاملة ناجحة (25 ، 50٪) بنسبة كفاءة عند 74 ، 59.6٪ على التوالي. تثبت النتائج أيضًا أن استخدام خليط الأعشاب يعزز التعداد الميكروبي في منطقة الجذور والذي من المتوقع أن يساعد في امتصاص و/أو تحلل الهيدروكربونات البترولية لمدة تصل إلى 52 يومًا. على الرغم من الحاجة الى وقت أطول يوفر هذا المزيج من الحشائش خيارًا صديقًا للبيئة وايضا فعالًا من حيث تكلفة إدارة بعض مواقع النفايات فبالتالي هناك حاجة ماسة لمزيد من الدراسات في مجال المعالجة النباتية.

INTRODUCTION:

The use of sustainable technologies to remediate petroleum drilling waste has attracted a greater attention and is a proper option for Libya, where the infrastructure and resources limit the feasibility of using conventional treatment strategies. Currently in Libya, and similarly in most of developing countries, there are various challenges that prevent the use of physical and thermal disposal techniques. (Ehmiada, 2008). However, overcoming oil sludge pollution can be achieved physically, chemically and biologically (Gong *et. al*., 2018; Roudneshin and Azadeh, 2019). Due to its lower cost and the production of non-toxic, environmentally friendly products, bioremediation has been used as an alternative to treat environmental pollution. One of the sustainable and powerful bioremediation techniques is phytoremediation. It involves the use of vegetation to remove and control wastes, or to increase waste break down, via the action of the microorganisms that are present in the plant's rhizosphere, through phytotransformation, rhizosphere bioremediation, phytostabilisation, phytoextraction, phytovolatilization or rhizofiltration (McCutcheon *et. al*., 2003). Therefore, it has the potential to minimise the concentration, mobility and toxicity of contaminants in contaminated media (Wuana and Okieimen, 2011). The most important factors affecting the success of phytoremediation and the time required to treat contaminated soils are plant type and the addition of soil amendments and/or bulking agents (Rhykerd *et. al*., 1999; El-Dars *et. al*., 2016; Shrestha *et. al*., 2019). Phytoremediation is most effective at sites where contamination by organic materials is not too substantial. Among the various mechanisms involved in phytoremediation of contaminated soils, rhizodegradation is of greatest importance for the removal of hydrocarbons from soil (Asim *et. al*., 2015; Dhote *et. al*., 2017). Rhizodegradation is enhanced by the presence of plant root exudates including enzymes, flavonoids, organic acids, sugars and amino acids. They play an important role in the growth of bacteria and fungi in the rhizosphere, leading to the degradation and mineralization of hydrocarbons pollutants (Reichenauer and Germida, 2008; Dadrasnia and Ismail, 2015). Several plants have already been identified and trialled for rhizoremediation of organic compounds, including wild grass species such as Bermuda grass (*Cynodond actylon*), tall fescue (*Festucaarundinacea Schreb*), and star grass (Ponterderiaceae) (Anyasi and Atagana, 2018). All aforementioned grasses have fibrous root systems, resulting in large root length and surface area per unit volume of surface soil, the fibrous roots provide a larger surface area for colonization by microorganisms than a taproot plant, which allow greater interaction between rhizosphere microbial community and the contaminant (Wang *et. al*., 2011). The main objective of this study was to assess the ability of commercial recommended and local use of mixture grass to grow in soil contaminated by drilling waste that contains petroleum hydrocarbons. Moreover, consider the feasibility of adopting this grass in Libya for the phytoremediation of drilling waste and stabilising contaminated soils.

MATERIAL AND METHODS:

Uncontaminated soil was collected from Al-Ameria village, about 40 Km southwest of Tripoli city at location (*E: 12° 57' 09", N: 32° 38' 91"*). They were then sieved to <4 mm to eliminate stones and plant material and thoroughly mixed to homogenise. Samples of drilling waste were collected during drilling operations in Jalu in the Libyan Desert. Then the samples were placed in plastic containers for transporting to the laboratory. To eliminate coarse rock and plant material, the samples were exposed to a careful hominization, air drying for two weeks and thoroughly mixed to ensure uniformity. Then finally sieved through a 2 mm mesh. Soil texture was determined using the hydrometer method as described in Barman and Choudhury (2020).

To assess the toxicity of drilling waste, leaching procedure were carried out according to British Standard BS EN 12457-1:2002. Particles of solidified waste <4 mm were used in the assay and they were exposed to 10 times its volume of deionised water and agitated for 24 hours under defined conditions. Then, the residue was separated initially by filtration using Whatman filter paper followed by membrane filtration (0.45 μm). Finally, the properties of the eluate were measured using standard methods for water analysis (GC-FID). Gas chromatography, flame ionization detector was used to identify and quantify total petroleum hydrocarbons (TPHs) originated from the samples containing drilling waste, and to evaluate the performance of a selected waste treatment scenario by measuring TPHs in waste leachate according to EPA5201A.

The pH of waste samples, native soil and leachate was measured according to British standard BS EN 15933:2012 using a pH meter (BOECO Multiparameter Model MBT-700, Germany). Electrical conductivity (EC) of raw materials and solidified samples were measured according to ASTM D1125 using a conductivity cell (BOECO Multiparameter Model MBT-700, Germany) by measuring the electrical resistance of a

1:5 solid: water suspension. Solution from leachability tests were measured by immersion of the conductivity cell in the solution. Flame photometer (FP-640B, Germany) and UV-Vis spectrophotometer GENESYS (Thermo Fisher Scientific, UK) were used to measure the concentration of extractable K and Total P in waste samples and soil. Modified method of Kjeldahl was used to determine the total N concentration as described in (TC WI, 2003).

The pot experiment was conducted in an outdoor investigation experiment between April 23rd and June 14th for 52 days. The experimental design utilized two different soils; control (C, native uncontaminated soil), which has been previously air-dried and sieved as described above and soil contaminated with water-based drilling waste (water based mud (WBM)). Different proportions of native added to the contaminated soil (750 g in total) using different dilution factors shown in table 1 was added to plastic pots with radius 9 cm and height 13 cm. Each treatment was replicated 4 times, giving a total of 16 pots.

Table 1: Description of pot experiment design

Code	Treatment	Dilution Factor	TOC wt. %	TOC mg/kg
C	Control (Native Soil)	**1**	**0.016**	**160**
25-W	25 WBM:75 Native Soil	**4**	**0.12**	**1244**
50-W	50WBM:50 Native Soil	**2**	**0.25**	**2488**
75-W	75 WBM: 25 Native Soil	**1.33**	**0.37**	**3732**

A recommended mixture of grass species of (*F. arundinacea*, *P. pratensis* and *L. perenne*) from Semiorto seeds (Italian company) was selected as the plant species due to its ability to grow in the harsh conditions of temperature, light and drought experienced in the Libyan Desert. The viability of these seeds was tested using the floatation technique. This was done by soaking the seeds in distilled water for 5 min. Thereafter, all floating seeds were discarded as non-viable and seeds that sank are now considered viable. Before seeds are planted, they were then surface sterilized with a 10% hydrogen peroxide solution. According to preliminary data, the optimum germination percentage of the grass in native soil required seeding was 84%. Then the Seeds sawed to a depth recommended in pots contain native soil (control), and deferent soil contaminated by WBM. A composite NPK fertilizer (of N: P_2O_5: K_2O of 10: 8: 9) prepared from high purity potassium nitrate and phosphate salts (Fisher Scientific UK) was applied to enhance the natural fertility of the soils. The pots were then arranged in randomized complete block design and placed outdoor for 52 days. The samples were labelled according to the concentration of the WBM as follows: 0% WBM is a control sample (C), 25% WBM is 25-W, 50% WBM is 50-W and 75% WBM is 75-W. During the time of the assay, the temperature ranged between 23 to 37ºC, and humidity between 40-60%. Lighting was natural sunlight with a light/dark cycle of approximately 14/10 hr. Irrigation water was added to achieve 30% of the water holding capacity of each treatment (c. 0.1 ml g^{-1}) to avoid leaching of hydrocarbons. After 52 days, the plants were carefully extracted from each pot and divided into roots and shoots. Both parts were placed sealed in plastic bags and stored in a cool place until analysis.

Samples for TPHs analysis by GC-FID were extracted. Two grams of un-planted soil/waste mixtures from all treatments, and rhizosphere soil from planted treatments mixed thoroughly with 3 g of anhydrous sodium, then 10 ml of dichloromethane was added. The samples were shacked for 15 and then centrifuged at 4000 rpm for 20 min. The supernatants transferred to amber glass vials and stored at 4ºC until analysed, using GC-FID. Leachates were serial extracted using 2 x 5 ml dichloromethane in a separation funnel by allowing the aqueous and organic phases to separate, then removing the lower (organic) fractions through anhydrous sodium sulphate and combining them, they were concentrated to 5 ml using a rotary evaporator. All extracts were analysed by GC-FID according to the method described above.

Total organic carbon (TOC) was quantified using a Shimadzu Flash EA 1112 Nitrogen and Carbon analyser. Leachate samples were acidified, thereafter, TOC was determined using a Shimadzu TOC-Vcph carbon analyser.

Grass heights (cm) were measured every four days. Total biomass (wet weight) of shoots and roots was measured at the end of experiment. To evaluate the microbial activity, two techniques were used. First, Oxidase disc (Liofilchem®, Italy) was used. In this technique, the rhizosphere soil extract solutions from each treatment and the control sample were dropped on the disc separately and changing the colourless dye to a violet colour was the indication of microbial respiration. Second, the measurement of dehydrogenase activity (DHA) was used as an indicator of biochemical reactions that taking place in the rhizosphere, and linked to redox process needed for oxidation of the hydrocarbons present (Utobo and Tewari, 2014). The colourless water-soluble dye triphenyltetrazolium chloride (TTC) used as an indicator of electron transport system (ETS) activity. In the presence of dehydrogenase enzymes TTC is reduced to insoluble red coloured triphenyl formazan (TF) as described by Sun *et. al*. (2014).

Compact DryTMCF Test (NISSUI, Japan) was also used to count the heterotrophic bacterial population of the soil samples for each treatment, and the control soil sample. This was done as follows: 1 g of soil sample was weighted, and then serial dilutions of 1:10, 1:10^2, 1:10^3 and 1:10^4 were prepared for each treatment using phosphate buffer (pH 7.2). 1 mL of aliquots from each dilution was incubated onto the test dish at 35 °C ± 2 °C for 24 h. The number of viable total heterotrophic rhizobacteria in the soil samples was calculated from the number of colonies formed according to the manufacturer's instructions.

IBM SPSS Statistics version 24 software was used to analyse the data using standard analysis of variance (ANOVA). Both ANOVA with Welch Howell of F-ratio correction, and Games-Howell test for post-hoc multiple comparisons, and Nonparametric Kruskal-Wallis H test were performed. Probability values of 0.05 or less ($P \leq 0.05$) were taken to be significant.

RESULTS AND DISCUSSION

Soil and waste analysis

The physical and chemical analysis of the soil and drilling waste contaminated soil prior to the experiment is given in table 2. Native soil samples have a sandy texture with a particle-size distribution of (89.38% sand, 8.6% silt, and 2.02% clay). Although the soil samples were collected from barren land that has not been fertilized or cultivated, the results indicate a high nutrient content of (N and P). A possible explanation for this might be that even if it was never exploited, the geological nature of the soil of the Jafara Plain region contributed to these concentrations (Zurqani, 2021). The results, as shown in Table 2, indicate that the waste sample has high petroleum hydrocarbon content and is considered hazardous waste when compared to the UK landfill acceptance criteria for granular waste acceptable at landfills (Council Decision annex 2003/33/EC).

Table 2: Physical properties, chemical composition of soil and WBM samples

Test	Units	WBM	Native Soil
pH	**-**	**8.4**	**8.43**
EC	**mS.cm^{-1}**	**4.06**	**0.07**
TDS	**mg.kg^{-1}**	**2035**	**40**
Organic matter (LOI)	**mg.kg^{-1}**	**21.3**	**0.0**
K	**mg.kg^{-1}**	**1279**	**164.0**
Total N	**mg.kg^{-1}**	**0.8**	**14367**
Total P	**mg.kg^{-1}**	**0.6**	**73.55**
TOC	**Wt.%**	**0.5**	**0.016**

Influence of Soil Properties

During irrigation, it was observed that with the increase in the concentration of WBM, the resistance of the irrigation water infiltration was increased, as it was at the highest concentration (75-W) and instead ponded water on the surface of the pot. As these are sandy soils, this is undoubtedly attributed to the TPHs content and the hydrophobic nature of these compounds, which will repel water intrusion and result in a dry subsurface layer of the soil. Subsequently, will affect the bioavailability of nutrients, which govern plant growth in those treatments. In contrast, water penetration in the other treatments (control, 25-W and 50-W) was significant and reasonable grass growth was observed.

Growth and biomass measurements

All measures of plant growth (shoot biomass and height, root biomass) indicated the tolerance of the grass mixture to grow in drilling waste was limited, with the grass only able to tolerate TPHs concentrations below 2488 mg kg^{-1} TOC. The grass did show a promising ability to grow in soils contaminated by water-based drilling waste at a low level of TOC (25% WBM). The growth was lower at a medium level of 50% WBM (Figure 1). However, the Attempts to grass seed germination in mixture containing 75% WBM were unsuccessful, thus these systems will not be considered further. There was a noticeable difference in seed germination time between the different treatments and the control sample, as germination occurred after 8 days of sowing in the control sample, while it occurred after 10 and 13 days in 25-W and 50-W samples, respectively. This result may be explained by the fact that TPHs pollution in the soil inhibited seed germination when the concentration of petroleum increased (Tang *et. al*., 2011).

C 25-W 50-W

Figure 1: Variation in grass growth over the experimental period in soils

Grass growth in the 50 % WBM contaminated soil (50-W) had a substantial decrease in shoots and roots biomass (Figure 2 and 4) and grass height (Figure 3) comparing to the control.

The total shoots biomass of the control was significantly higher than 25-W and 50-W, $p < 0.001$, however, there are no significant differences in biomass between 25-W and 50-W treatments, $p = 0.261$ (Figure 2).

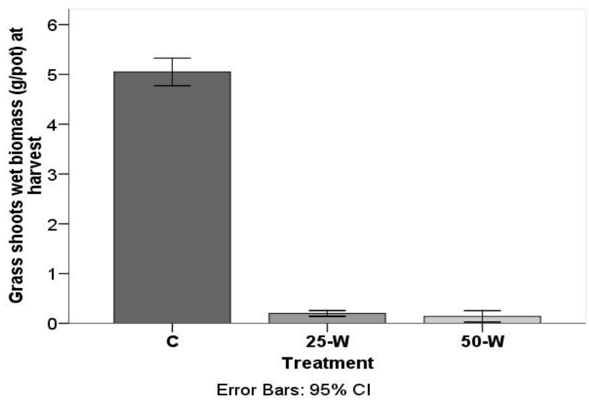

Figure 2: Total shoots biomass (wet weight) at harvest

Overall plant height decreased significantly with increasing contamination level, $p = 0.05$. The most significant difference was observed between control and 50-W, $p = 0.027$ with an average height of 13.3 and 4 cm, respectively (Figure 3).

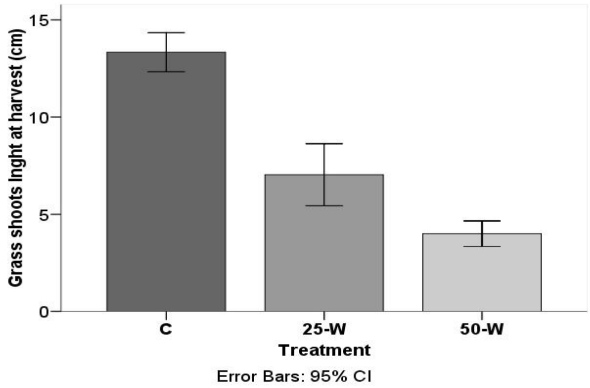

Figure 3: The average height of the grass at harvest

Root density is considered as an important factor for the phytoremediation of TPHs (Suelee *et. al*., 2017). Root biomass varied between treatments (Figure 4). The reduction in root biomass compared with the control treatment was significant, p =0.05 and 0.027, respectively. Greater concentrations of TPHs appear to inhibit the development of the root system and therefore growth. The Inhibition effects may result from the petroleum hydrocarbons prevent the grass seed germination through direct and/or indirect effects. Direct effects caused by the toxicity of petroleum hydrocarbons and their capability to break through the plant cell surface, and solubilize the lipids of the cell membranes, leading to tissue desiccation and death, as suggested by Vaughn and Holser, (2007). Indirect effects resulted from the hydrophobic properties of petroleum hydrocarbons and their ability to coat the seeds and restricting H_2O and O_2 uptake and/or reducing the oxygen concentration in the root zone, which causes a reduction in root elongation and density, proliferation, viability, respiratory capacity, carbohydrate accumulation, hormone synthesis, and nutrient uptake, as reported by Schnoor, 1995; Huang and Scott, 1999; White *et. al*., 2003; Merkl *et. al*., 2005).

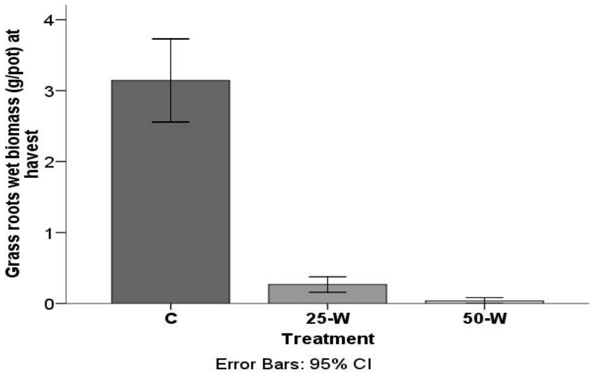

Figure 4: Reduction in total roots biomass (wet weight) at harvest

The results also show that the root networks in the 25-W treatment are distinctly larger, longer more fibrous and had a greater surface area than those in the 50-W treatment (Figure 5). This would be expected to reflect positively on the ability of the grass to phytoremediation of hydrocarbons.

Figure 5: Reduction in root density of the grass at harvest

The results of the chlorophyll content agreed with the previous results (Figure 6). There was a positive trend in the chlorophyll content of the grass that is grown in uncontaminated soil while it was a slight increase in the chlorophyll content of the 50-W sample. Surprisingly, the content was slightly less in 25-W than in 50-W. This is rather contradictory result, and it may be because some plants successfully resist soil contamination by petroleum hydrocarbons, and they can survive soil contamination of about 18,000 mg kg^{1-}by metabolic changes, leading to the reduction of the destructive effects of petroleum hydrocarbons (Rahbar *et. al*. 2012).

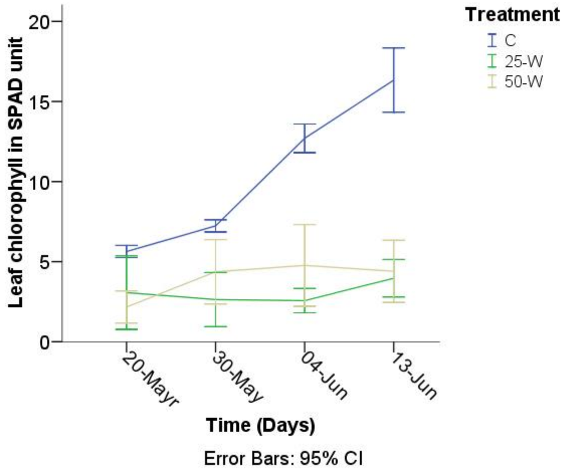

Figure 6: The variation in chlorophyll content of the grass

Soil enzymes activity assays

To assure the correct sequence of biochemical reactions in the planted treatments, the soil dehydrogenase enzyme activity DHA was examined after 52 days of the phytoremediation experiment. The qualitative measurement (Present/absent) that were carried out for all treatments confirmed the presence of DHA activity in treatments at 25% and 50% following cultivation with a selected mixture of grass (Quilchano and Marañón, 2002; Salazar *et. al*., 2011). Similarly, Oxidase discs results were positive for all treatments, with more increasing in the dye colour when treated with 25-W and 50-W.

Total heterotrophic rhizosphere bacterial count

The obtained results at the end of study showed that the bacterial count was higher by orders of magnitude in the amended drilling waste planted treatments. The data also confirmed that the growth of the selected grass in amended drilling waste significantly increased the number of oil-degrading bacteria in the root zone for treatments 25% and 50%.

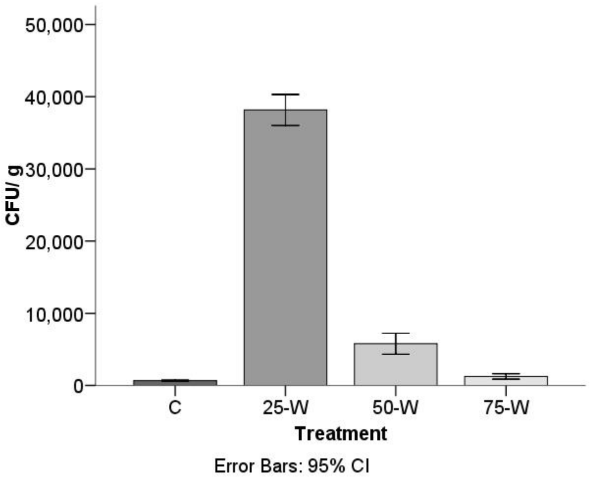

Figure 7: Total heterotrophic rhizospheric bacterial count

The results in the figure 7 illustrated that there was an increase in biodegradation of the bacteria in the microbial community, during the treatment in the rhizosphere soil in all planted soil samples. Overall, the difference in heterotrophic bacteria count was significant, $p < 0.0001$, with an exception between the control and 75% treatment, $p = 1$. The obtained data corresponds with findings in similar studies that have assessed the microbial counts in contaminated soils (Mcintosh *et. al*. 2015; Varjani and Upasani, 2017). These studies confirmed that the addition of plants and soil amendments increases bacterial counts compared to unplanted soils, and also confirmed that the examined grass mixture could tolerate the contamination levels in amended drilling waste to a level lower than (2488 $mgKg^{-1}$ TOC). In addition, the rhizosphere soil in planted samples contains survived organisms can tolerate the stress such as TPHs contamination, heat and the calcareous nature of drilling waste. Moreover the examined mixture of grass will be effective, in terms of bioremediation of petroleum hydrocarbons through a rhizosphere effect.

Effect of TPHs on phytoremediation by a mixture of grasses

Planting of a selected mixture of grass in amended drilling waste, increased the removal of petroleum hydrocarbons from drilling waste, when the total organic carbon concentration was below 0.25 wt. % (2488 $mgKg^{-1}$) after the bioremediation treatment for 52 days (Figure 8).

The petroleum hydrocarbon concentration in each successful treatment (25W and 50 W) after 52 days were removed at 74 %, and 59.6 %, respectively, and compared to their respective original soil containing petroleum hydrocarbon of 1244 $mgKg^{-1}$ and 2488 $mgkg^{-1}$, respectively. The analysis of soil leachate indicates that the petroleum hydrocarbons were reduced by 74% when treated with 25% (from 1244 $mgKg^{-1}$ to 250 $mgKg^{-1}$), and70.3% when 50% (from 2488 $mgkg^{-1}$ to 746 $mgKg^{-1}$) used. Light aliphatic (C10-C16) and aromatics (C8-C21) were reduced to the accepted limit used to consider the treated drilling waste as inert waste (Less than 1000 $mgKg^{-1}$).

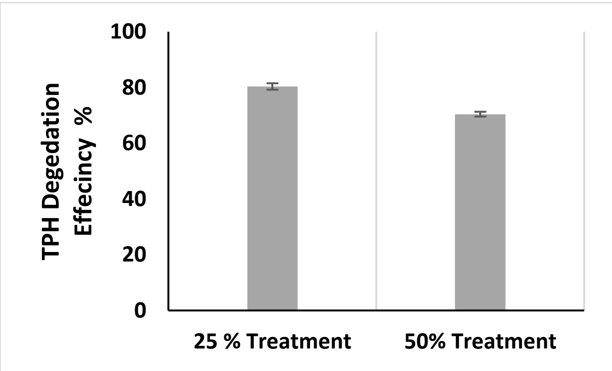

Figure 8. Efficiency of grass in degradation of petroleum hydrocarbon from contaminated soil

The results in the figure 8 indicate that the selected mixture of grass can efficiently survive and tolerate petroleum hydrocarbons below (0.25 wt. % or 2488 $mgKg^{-1}$ TOC), and that is a proof of that the use of grass mixture enhances the microbial enumeration and diversity in the root zone, which is expected to help in uptake and/or degradation of petroleum hydrocarbons.

Conclusion:

In summary, it is clear that the studied mixture of grass has highly significant positive effect on degradation of petroleum hydrocarbons within the contaminated soil, and would be recommended as a promising technique in the suit of in-situ remediation technologies, that adapt the Libyan climate. The Studied mixture of grass has not shown any signs such as wilting, lodging or defoliation during the treatment period for 52 days. Only exception was the reduction in shoot and root biomass, when exposed to petroleum hydrocarbon with TOC content for 2488 $mgkg^{-1}$ or higher. Furthermore, the studied mixture enhance the growth of bacteria in the root zone which enhance the dehydrogenase enzymes activity DHA which is tightly linked with microbial oxidation-reduction processes.

Future studies still are needed to indicate more precisely the phytoremediation mechanisms used by a mixture of grass species. Analysing hydrocarbons speciation in plant tissue (Roots and shoots). In addition to hydrocarbons speciation in soils in intervals for long periods, and after harvesting the above-ground part of the plant many times can provide a better understanding of the phytoremediation mechanisms used by a mixture grass to dissipation of petroleum hydrocarbons.

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REFERNCES

Anyasi, R. and Atagana, H. (2018). Profiling of plants at petroleum contaminated site for phytoremediation. International Journal of Phytoremediation, 20(4), pp.352-361.

Asim Shahzad, A. (2015). Rhizodegradation of Hydrocarbon from Oily Sludge. Journal of Bioremediation & Biodegradation, 06(03), pp.2-11

Barman, U., & Choudhury, R. D. (2020). Soil texture classification using multi class support vector machine. *Information processing in agriculture*, *7*(2), 318-332

Dadrasnia, A. and Ismail, S. (2015). Bio-Enrichment of Waste Crude Oil Polluted Soil: Amended with Bacillus 139SI and Organic Waste. International Journal of Environmental Science and Development, 6(4), pp.241-245.

Dhote, M., Kumar, A., Jajoo, A. and Juwarkar, A. (2017). Assessment of hydrocarbon degradation potentials in a plant–microbe interaction system with oil sludge contamination: A sustainable solution. International Journal of Phytoremediation, 19(12), pp.1085-1092.

Ehmiada 2008. Developing an environmental management approach to Libya's upstream petroleum industry. Sheffield Hallam University-Sheffield UK.

El-Dars Farida M.S. Sayed A. El-Tohamy, Islam Abdelnaser Abdelhafeeze. (2016) Rmoval of total petroleum hydrocarbon from contaminated soil via phytoremidation and amended with sungarcane bagasse. J. Biol. Chem.Environ. Sci., Vol. 11(2): pp.283- 293

GONG, Z., WANG, L., WANG, Z., WANG, Z., XU, Y., SUN, F., SUN, Z., LIU, Z. & ZHU, L. 2018. Experimental study on combustion and pollutants emissions of oil sludge blended with microalgae residue. *Journal of the Energy Institute,* 91**,** 877-886.

Huang B, Scott NeSmith DS. (1999). Soil aeration effects on root growth and activity. Aca Hort. (ISHS). 504: pp.41–52

McCutcheon, S. and Schnoor, J. (2003). Phytoremediation. Hoboken, N.J.: Wiley-Interscience.

Mcintosh, P., Kuzovkina, Y., Schulthess, C. and Guillard, K. (2015). Breakdown of low-level total petroleum hydrocarbons (TPH) in contaminated soil using grasses and willows. International Journal of Phytoremediation, 18(7), pp.656-663.

Merkl N, Schults-Kraft R, Infante C. (2005). Assessment of tropical grasses and legumes for phytoremediation a of petroleum-contaminated soils. Water Air Soil Pollut. (165), pp.195–209

Quilchano, C. and Marañón, T. (2002). Dehydrogenase activity in Mediterranean forest soils. Biology and Fertility of Soils, 35(2), pp.102-107.

Rahbar, F. G., Kiarostami, K., & Shirdam, R. (2012). Effects of petroleum hydrocarbons on growth, photosynthetic pigments and carbohydrate levels of sunflower. Journal of Food, Agriculture & Environment, 10(1 part 2), 773-776.

Reichenauer, T. and Germida, J. (2008). Phytoremediation of Organic Contaminants in Soil and Groundwater. ChemSusChem, 1(8-9), pp.708-717.

Rhykerd R.L., Crews B., Mclnnes K.J., Weaver R.W. (1999). Impact of bulking agents, forced aeration, and tillage on remediation of oil-contaminated soil. Bioresource Technology (67), pp.279-285

ROUDNESHIN, M. & AZADEH, A. 2019. A novel multi-objective fuzzy model for optimization of oil sludge management by considering Health, Safety and Environment (HSE) and resiliency indicators in a gas refinery. Journal of Cleaner Production, 206, 559-571.

Salazar, S., Sánchez, L., Alvarez, J., Valverde, A., Galindo, P., Igual, J., Peix, A. and Santa-Regina, I. (2011). Correlation among soil enzyme activities under different forest system management practices. Ecological Engineering, 37(8), pp.1123-1131.

Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH. (1995). Phytoremediation of organic and nutrient contaminants. Environ Sci Technol. 29, pp.318–323

Shrestha, P., Bellitürk, K. and Görres, J. (2019). Phytoremediation of Heavy Metal-Contaminated Soil by Switchgrass: A Comparative Study Utilizing Different Composts and Coir Fiber on Pollution Remediation, Plant Productivity, and Nutrient Leaching. International Journal of Environmental Research and Public Health, 16(7), p.1261.

Suelee, A.L., Hasan, S.N.M.S., Kusin, F.M. (2017). Phytoremediation Potential of Vetiver Grass (Vetiveria zizanioides) for Treatment of Metal-Contaminated Water. Water Air Soil Pollut228: 158.

Sun S, Guo Z, Yang R, Sheng Z, Cao P. 2014. Study on the triphenyl tetrazolium chloride-dehydrogenase activity (TTCDHA) method in determination of bioactivity for treating tomato paste wastewater. African Journal of Biotechnology, 11, 7055–7062.

Tang, J., Wang, M., Wang, F., Sun, Q., & Zhou, Q. (2011). Eco-toxicity of petroleum hydrocarbon contaminated soil. Journal of Environmental Sciences, 23(5), 845-851.

Utobo E.B., Tewari L., 2014. Soil enzymes as bioindicators of soil ecosystem statuts. Appl. Ecol.Environ. Res. 13(1), pp.147-169.

Varjani, S. and Upasani, V. (2017). A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. International Biodeterioration& Biodegradation, 120, pp.71-83.

Vaughn, Holser A. (2007). Evaluation of biodiesels from several oilseed sources as environmentally friendly contact herbicides. Industrial Crops and Products pp. 63–68

Wang, H., Li, C., Peng, Z. and Zhang, S. (2011). Characterization and thermal behaviour of kaolin. Journal of Thermal Analysis and Calorimetry, 105(1), pp.157-160.

White, PM Jr, Wolf DC, Thoma GT, Reynolds CM. (2003). Influence of organic and inorganic soil amendments on plant growth in crude oil-contaminated soil. Int J Phytorem. pp. 381–397.

WI, TC. "Determination of Kjeldahl Nitrogen in soil, biowaste and sewage sludge." (2005).

Wuana, R. and Okieimen, F. (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecology, 2011, pp.1-20.

Zurqani, H. A. (Ed.). (2021). *The Soils of Libya*. Springer.