Effect of Sewage Sludge Compostin Enhancingthe Growth of Grasses in Phytoremediation of Diesel Contaminated Soil

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Abstract
Contamination with diesel, which is a heavy petroleum fraction used as fuel in diesel engines is a concern in the Middle East. Distribution of these toxins to the environment causes many ecological and human health risks. The present study assesses the effect of sewage sludge compost (Kala compost, obtained from Haya Water Company (HW) in the Sultanate of Oman)on the phytoremediation efficiencies of selected grasses. The effect of compost on rhizoremediation of petrogenic hydrocarbons was studied.A pot culture study was conducted in a diesel contaminated soil brought from Barka, Oman, with nine treatments and four replications. The study was done in a greenhouse in the experimental farm attached to the Sultan Qaboos University. Two levels of compost (10 % and 20%) and two grasses (Ryegrass and Bermuda grass) were tested in the study. The efficiency of compost along with plants in the remediation of total petroleum hydrocarbons (TPH) was assessed in terms of TPH concentration in soil and plants (root and shoot) at 90 days after planting. Application of 10% compost was found to be better than 20% for the phytoremediation of diesel contaminated soil.A significant reduction in total petroleum hydrocarbons was noticed by the growth of Bermuda grass(*Cynodondactylon*) compared to Rye grass (*LoliumPerenne*)and Natural Attenuation. Thus, the results revealed the promising effect of sewage sludge compost in contributing to the phytoremediation of petroleum hydrocarbons (TPH) in the diesel contaminated soils of Oman.

Keywords
Sewage sludge; Bermud grass; Ryegrass; TPH

INTRODUCTION

Hydrocarbon compounds are the majority common class of environmental pollutants reported in developed countries (Shahsavari *et al*., 2013). Petroleum hydrocarbon contamination is caused by drop out from oil production wells, leakage from fuel storage tanks, mining, shipping, refining and breaking of oil pipelines etc. (Saadoun and Al-Ghazawi, 2005; Chakrabarti and Ghosh, 2010). During Iraq and Iran war, vast quantities of oil spill (about 1000 tons) were discharged into the Arabian Gulf and released to the Kuwaiti desert (Saadoun and Al-Ghazawi,2005;Al Hinai,2013). In 1994, around 16,000 tons of Iranian crude oil was spilled in the Gulf of Oman and in 2008, huge quantity of oil was released when the oil transport ship was sunk in the Omani Gulf (http:// www.Cleanglobe. Eu/press220208.html, 2009). Plenty of petroleum products in the Sultanate of Oman create a threat of contaminating the environment through oil accidents resulting from spills. Thus, the dominance of petroleum products in the environment creates a condition for distributing large amounts of these toxins into populated areas and ecosystems. Diesel oil is a mixture of toxic and carcinogenic compounds such as straight and branched alkanes, cycloalkanes, monaromatics and polyaromatic aromatic hydrocarbons (PAHs) (Adam and Duncan, 1999; Komilis *et al*., 2010; Das and Tiwary, 2013). This type of oil may cause chronic or acute effects in human beings, plants and animals (Racine, 1994). The requirement for effective, inexpensive, and sustainable technologies for cleaning the petrogenic impacted environments is raising day by day (Gaskin and Bentham, 2010). Several studies have been conducted on the remediation of hydrocarbon polluted sites (Dott *et al*., 1995; Ibraham *et al*., 2013). But, most of them are physical like excavation and chemical technologies such as injection chemical oxidants to groundwater or polluted soil (Shahsavari, 2014) with associated negative impacts on the environment. Biological remediation methods like adding microbes and nutrients to degrade hydrocarbons from contaminated soils (Speight and Arjoon, 2012; Al-Hinai, 2013) are ecologically sound and feasible. Nevertheless,

phytoremediation, which involves plants and their associated microorganisms to remove or degrade contaminants, shows a promising skill for the remediation of hydrocarbon-contaminated soils (Chaudhry *et al*., 2005; Gómez-Sagasti *et al*., 2012; Phillips *et al*., 2012). Many reports have described the positive effects of the rhizosphere of grasses Ryegrass (*Lolium Perenne*) and Bermuda grass (*Cynodondactylon*) on petrogenic hydrocarbon bioremediation (Günther *et al*., 1996; Gaskin and Bentham, 2010). But the studies were conducted elsewhere and their effectiveness under the arid climatic conditions of Oman was not investigated till date. In phytoremediation, plants not only degrade organic contaminants straightly by their enzymatic activities, through a process called phytodegradation, but also stimulate the rhizosphere microbial community which can cause the degradation of organic pollutants by microorganisms, through a process called rhizoremediation or rhizodegradation (pillon-Smits, 2005). This can clarify the main path of hydrocarbon removal in the phytoremediation process (Hall *et al*., 2011).
In addition, organic amendments such as compost or sewage sludge can enhance phytoremediation for degrading hydrocarbon pollution in contaminated soil as they are useful to plant growth and supply nutrients and carbon sources to contaminated soil for microbial activity (Namkoong *et al*., 2002; Ghanem, 2013).

In Oman, new organic fertilizer (Kala compost) was introduced in 2010 from the mixing of sewage sludge and green waste consists of grasses, trees. The new fertilizer is produced by Haya Water Company (HW) which is the modern wastewater company in Oman and established its commercial operation in 2006. This manure will be used in this study to examine its efficiency in enhancing phytoremediation process of diesel contaminated soil.
Two levels of compost (10 % and 20%) and two grasses (Rye grass and Bermuda grass) were tested in the present study. The efficiency of compost along with plants in the remediation of total petroleum hydrocarbons (TPH) was assessed in terms of TPH concentration in soil and plants (root and shoot) at 90 days after planting.

The aim of this study is to assess the ability of selected two plant species (Bermuda grass and Rye grass) to survive and grow in diesel-contaminated soil, and their potential use in rhizoremediation of petroleum hydrocarbons. And to study the effect of kala compost in enhancing the process of phytoremediation.

2. MATERIAL AND METHODS

A greenhouse pot experiment was conducted at Agricultural Research Satiation of Sultan Qaboos University (SQU). The experiment was conducted from March 2014 to June 2014. 36 plastic pots (12X14) were filled with 2 kg soil after placing a layer of gravel at the bottom of the pot. Table 1 gives the treatment details of the experiment.

After filling pots with 2 kg soil, 20 seedlings (15 days after sowing seeds) of P1 (Bermuda grass) and P2 (Ryegrass) were transplanted as per the treatments detailed above in Table 1. Since there were 9 treatments and 4 replications, 36 pots were there altogether. Four pots contained only contaminated soil without plants in them (control pots). The pots were kept in a green house with polyethylene transparent covering roof.

The plants were irrigated once a day with tap water (pH= 6.5 EC= 900 µs/cm) at levels that no run out from the bottom of the pots was there. Equal amount of water was added to all pots. The compost used in this study was Kala compost obtained from Haya Water Company (HW) with the chemical composition as follows:

pH 7.6, EC 31mS/cm , Total N2.6-2.8, Organic Carbon 28.04, Pb0.94mg/l, Cu <0.0004, Zn 2.22mg/l, Cd <0.001, Fe 7.29mg/l , Cr 0.51mg/l, Ni 0.50mg/l

Soil and plant analyses were carried out at the (SQU) soil laboratory in order to investigate the remediation of pollutants by plant growth.

Table 1. Treatment details

Treatments	Details
T1	C (contaminated soil alone)
T2	Contaminated soil + P1 (Bermuda grass)
T3	Contaminated soil + P2 (Rye grass)
T4	Contaminated soil+10% of compost +P1
T5	Contaminated soil+10% of compost +P2
T6	Contaminated soil+20% of compost +P1
T7	Contaminated soil+20% of compost +P2
T8	Non-contaminated soil (Background soil)+P1
T9	Non- contaminated soil (Background soil) + P2

2.1 Soil analysis

The analyses of contaminated and clean soil were done at SQU laboratory. Before the start of the experiment, contaminated soil as well as clean soil were analyzed for soil texture, pH, EC, Organic Carbon, Total Nitrogen, Total Petroleum Hydrocarbons (TPH) . Plant samples (both root and shoot) were analyzed for TPH. The methods used for the analyses are detailed in Table 2.

2.1.1 Soil Measurements after Harvesting

Soil samples after harvesting were kept in labeled plastic bags, and then kept in the refrigerator for avoiding volatilization of hydrocarbons. Analyzing soil samples for TPH, pH, EC were carried out by the same methods outlined in Table 2.

Table 2. Methods for Soil and Plant analysis

Parameter	Method	Reference
Mechanical composition of soil (sand, silt, clay)	Hydrometer	Klute,1986
Soil texture	Soil triangle	Brady, 1984
pH	Saturation Paste	United States Salinity Laboratory Staff, 1954
EC	Saturation Paste	United States Salinity Laboratory Staff, 1954
TPH	Gas Chromatography EPA method 1664	US EPA 2010
Moisture content	Oven drying	United States Salinity Laboratory Staff, 1954
Organic Matter (TOC)	TOC Analyser	AlKhamisi,2013
Total Nitrogen	Kjeldahl	AlKhamisi,2013
Plant analysis	Method	Reference
TPH	Gas Chromatography EPA method 1664	US EPA 2010

2.2 Plant Harvesting, Sampling and Analysis:

The plant samples collected (both root and shoot) were placed in separate labeled plastic bags. The plant samples were washed with tap water to remove soil particles stuck on the roots. Then, they were kept for further dryness in room temperature for about two days. Later, the plant’s roots and shoots were cut into small pieces using a scissor and weighted separately. Finally, the roots and shoots were kept in the paper bags and placed in an oven at 60^{o}C for drying and to grind them into powder for analysis of TPH.

2.3. Statistical Analysis:

The data for pH, EC and TPH were subjected to one-factor Analysis of Variance (ANOVA), using the computer software JMP (SAS Institute Inc, 2013)

4. Results and discussion:

The basic characteristics of the background soil (BG) as well as contaminated soil used in this study are presented in Table 3.

Table 3. Physical and chemical characteristics of the contaminated soil and BG soil used in the current study.

Soil	pH	EC (dS/m)	Texture	Total Organic Carbon (TOC)	Total Petroleum Hydrocarbon(TPH)
Contaminated soil	7.9	3.5	Loamy sand	10.5%	1.41%
Background soil (BG soil)	8.0	1.3	Loamy sand	1.8%	0.061%

4.2 Growth of Plants:

The performance of plants was observed during the growth period, there was no undesirable effects were noticed. This revealed the tolerance of plants to the diesel contamination in soil, which is in line with the findings by Ghanem at al. (2013) that no effect of pyrene in leaves development by using Ryegrass, alfalfa and oil seed rape plants species. Further, addition of compost triggered the growth of both the plant species with 10% compost having a more positive effect on plant growth. Nonetheless, Bermuda grass showed a better growth than (*Lolium Perenne*) in both clean and contaminated soils.

Compost addition at 20% showed stunted growth with yellowish leaves for (*Lolium Perenne*) This maybe an expression of phytotoxicity of compost, which is in line to the findings by Kirk *et al*. (2005) when they noticed the stressed and stunted growth of Perennial Rye grass and alfalfa seeds in petroleum contaminated soil. The high salinity of Kala compost (31 mS/cm) may be the reason for the phytotoxicity expressed in plants grown in soils with 20% compost addition.

4.2.2Biomass of shoots and roots

Shoots Biomass

Shoot biomass showed high significant differences between all treatments for both plants ($P<0.05$). In the pots of contaminated soil with either plant, the mean shoot growth was recorded at 0.63g with (Bermuda grass), while mean shoot weight for (Ryegrass) recorded at 0.71g. Bermuda grass grown in contaminated soil with 10% compost addition recorded a mean shoot biomass of 12.60g/pot. Whereas, Rye grass grown with similar conditions recorded a mean shoot biomass of 0.77g/pot. Addition of 20% compost to the contaminated soil increased the mean shoot biomass of Bermuda grass at 13.63g and for Ryegrass at 1.16g. This is in line with reports by Ghanem *et al*. (2013) that compost addition increased the dry weights of shoots of plants grown in the soil contaminated with pyrene compared to the soil without amendments addition. The positive effect of compost on plant growth may be due to the improvement of pedogenic features as well as triggering soil biology by providing conductive conditions for their growth (Duong, 2013)

The mean values of shoot biomass are given in Figure 2below:

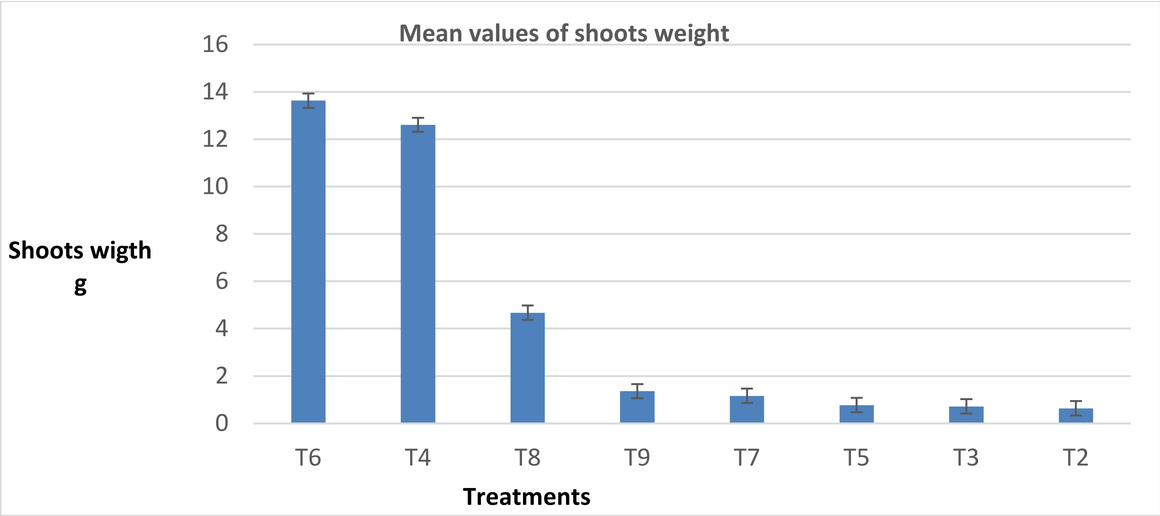

Figure 2 Mean values of shoots weight**.**

Roots Biomass

The growth of root biomass was highly significant by both plant species with in all treatments ($p<0.05$). The mean values of root biomass are given in Figure 3. The mean root biomass recorded in non-contaminated soil was 1.64g/pot for Bermuda grass (*Cynodon dactylon*), and 1.35g/pot for Rye grass (*Lolium Perenne).* When grown in contaminated soil amended with 10% compost Bermuda grass (*Cynodon dactylon*) recorded the highest root biomass of 15.97g/pot, whereas for the treatment conditions, Rye grass (*Lolium Perenne)*gave a mean root biomass of 0.4g/pot. Nevertheless, contaminated soil amended with 20% Kala compost gave low root biomass for Bermuda grass and for Rye grass at (3.87 and 0.66g/pot) respectively.

The observation of slow growth of roots by using Ryegrasses is consistence with the remarks of Kechavarzi *et al*., (2007) when they noted that Ryegrass prefers to grow in uncontaminated zones in their experiment before moving to diesel contaminated zones where the acceleration growth reduced. Moreover, Ghanem *et al*. (2013) noted that roots and shoots biomass of (*Lolium Perenne)* was adversely influenced by the presence of Pyrene in soil, and this may explain that the decreased root biomass of Rye grass (*Lolium Perenne)* in the present study is may due to the presence of diesel.

Overall, the improved growth of Bermuda grass in contaminated soil in terms of plant biomass consistencies with the findings of Razmjoo and Adavi (2012).

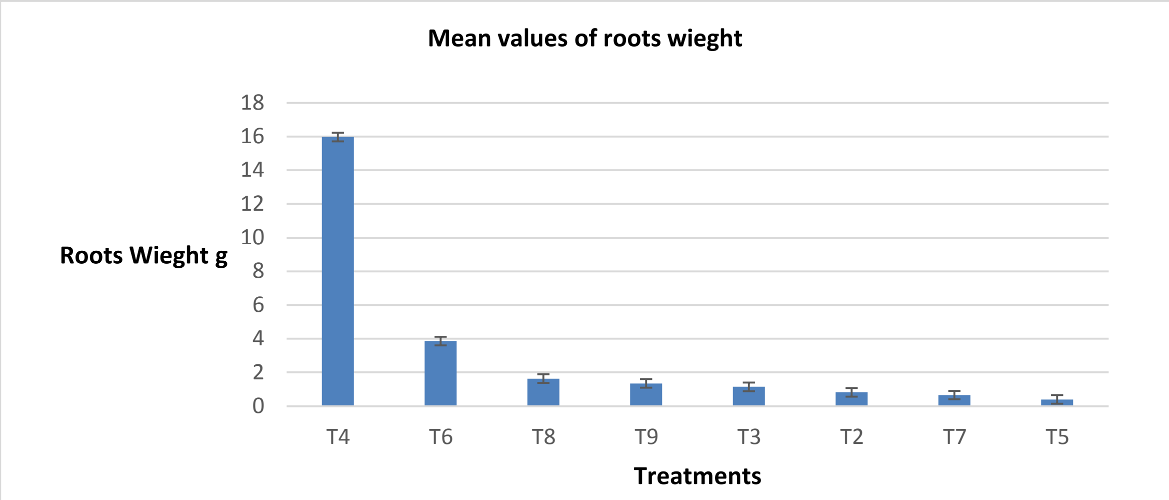

Figure 3 Mean values of roots weight

4.3 Removal of diesel fuel (Total Petroleum Hydrocarbons)

4.3.1 Instrumental analysis

In order to measure the content of diesel fuel (TPH %), hydrocarbons were extracted three times from (10g) of each soil samples with (10ml) hexane solvent. Hexane was used in this experiment according to (US-EPA Method 1664,2010; Dadrsina and Agamuthu, 2013). The solution was placed in ultrasonic bath for 30 minutes after dehydrated with Na_2SO_4 in (50ml) glass bottles, the first result of solution was filtered through nonabsorbent cotton in a funnel, then the procedure of extraction was repeated twice with adding (10ml) of hexane solvent each time, all the extracts were collected up to (30ml) in (100ml) weighted round flask and was evaporated completely using rotary evaporation equipment at 40^0C and 70 RMP rotations with providing 140 mbar vacuums. After that, the round flask which contained residual oil was placed in the dissector for further solvent evaporation, then its final weight was recorded and subtracted from its known weight at the beginning of the experiment, the result then was divided by the original weight of the soil sample to calculate TPH percentage gravimetrically.

4.3.2 Degradation of diesel fuel

The plant samples (both root and shoot were analyzed for TPH content. But the value obtained were negligible showing that the uptake of TPH by plants was not there. This reveals the fact that whatever TPH reduction in soil obtained was mainly by the degradation of hydrocarbons by the influence of plants as well as the microbes associated with them. This is consistent with the observation of Dadrasnia and Agamuthu (2013) when the reported no fuel remains in the plants in their study using different species of plants under different climates conditions. The reason attributed to the reduction of TPH in soil was through rhizodegradation mechanisms, mainly brought out by microorganisms which enhancing mineralization of oil in soil. The production of root exudates stimulated microbes in soil to degrade hydrocarbons.

The mean values of TPH% in soil with different treatments conditions are given in Figure 4:

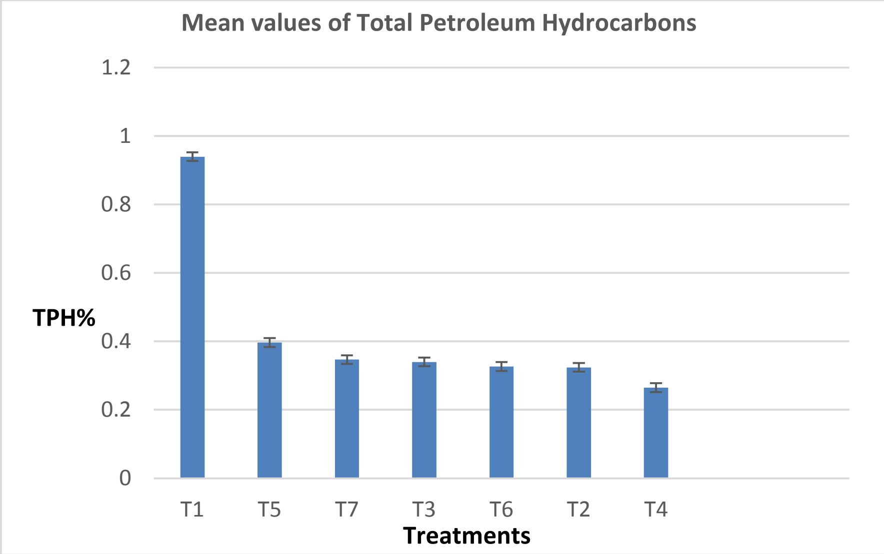

Figure 4. Mean values of Total Petroleum Hydrocarbons.

The statistical result shows that the removal of TPH in all treatments was highly significant ($P<0.05$). Degradation of hydrocarbon in contaminated soils showed that, in the non-vegetated pots with contaminated soil only (control), the mean removal of hydrocarbon was (17%) as shown in figure 5 indicating that diesel degradation was slow, may be because of low microbial count in the soil.There are previous reports that TPH in soil when left as such decreased very slowly because of no microorganisms to enhance degradation of diesel and thus hydrocarbons could stick to the soil particles (Ling and Isa, 2006) and the removal of oil was due to the evaporation route (Dadrasnia and Agamuthu,2011) throgh natural attenuation processe.

The mean removal of TPH in contaminated with Bermuda grass was at 71%, wherase for Rye grass it was almost 70%. This indicates that the capablities of these plants for degradion of hydrocarbons in diesel contaminated soil.

Application of 10% compost further enhanced the phytoremediation efficiency of these plants. The average removal of hydrocarbon was approximately at 77% and 65% in the contaminated soil amended with 10% compost when grown with Bermuda grass and Rye grass respectively.

However, in the pots of contaminated soil with 20% compost the removal of contamination was recorded at 71% and 70% when cultivated with Bermuda grass and Rye grass respectively. Application of 20% compost showed a lower degradation of TPH, when compared to the application of compost at 10%. Similar reports were there by Namkoong *et al*., (2002).

The high removal of TPH by the growth of plants may due to the root excretions as well as the microbial growth in the rhizosphere induced by the root exudates. High root biomass (9.6 g/pot) of Bermuda grass might be the attributing factor for high TPH degradation. The organic compounds released by the roots triggered the microbial growth in the rhizosphere, which stimulated the root contaminant interactions, favoring more degradation of TPH in soils. This was consistent with the observations made by Palmroth *et al*., (2002). There are reports that the activities of microorganisms in the rhizosphere degrade diesel through rhizodegradation mechanisms and the adding organic manures such as sewage sludge or compost can increase degradation of organic contaminants by supplying nutrients and carbon sources in contaminated soil (Namkoong *et al*., 2002).

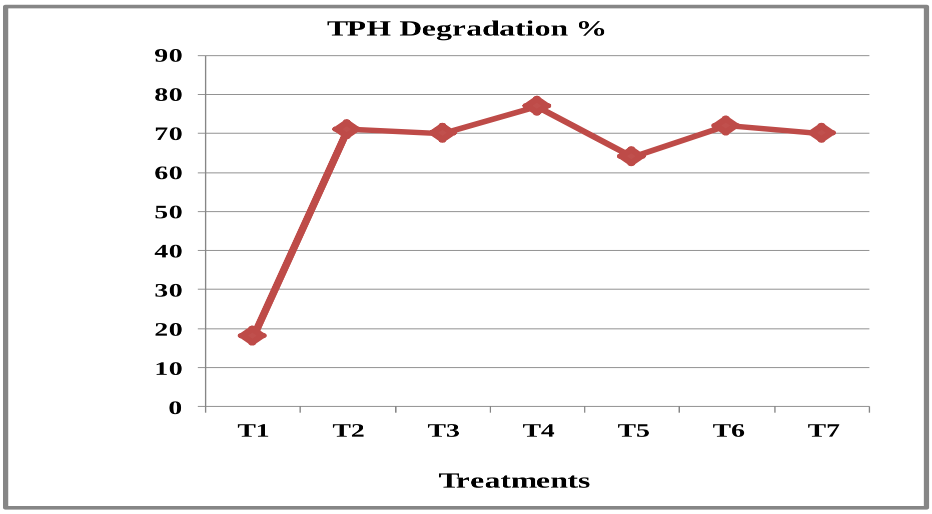

Figure 5. TPH degradation in each treatment

4.3.1TPH analysis

In order to calculate the degradation of diesel fuel by gravimetrical analysis the following equation was used:

Bioremediation% = Total TPH control sample - Total TPH of treated sample/Total TPH of control sample.

5. CONCLUSION

A significant reduction in total petroleum hydrocarbons was noticed by the growth of Bermuda grass, compared to Ryegrass and Natural Attenuation. Application of compost at 10% complemented the phytoremediation process. Rhizodegradation mechanism might be a consideration for enhancing the degradation of diesel fuel.

REFERENCES

Adam, G., Duncan, H. *Effect of diesel fuel on growth of selected plant species*. 1999. Environmental Geochemistry and health. 21(99),353-357.

Al-Hinai, M. 2013. *Effects of salinities and temperatures on bioremediation strategies for cleanup of oil polluted soils in Sultanate of Oman*. Master thesis.

Al-Khamisi, S.A.S. 2013. *Maximizing the use of Reclaimed water for crop production in Arid Regions*. PHD thesis.

Brady, N. 1984.*The Nature and properties of Soils*. Macmillan Publishing, New York.

Chakrabarti ,T. , Ghosh, T.K. 2010. *Bioremediation Technology*. Recent advances.

Chaudhry, Q., Blom-Zandstra, M., Gupta, S., &Joner, E. J. 2005.*Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organicpollutants in the environment.* Environmental Science and Pollution Research. 12(2005), 34–48.

Dadrasnia, A., Agamuthu, P. 2011. *Organic wastes to enhance phyto-treatment of diesel-contaminated soil*. J Waste Management and Research, 31(11) 1133-1139.

Dadrasina, A., Agamutu, P. 2013. *Dynamics of diesel fuel degradation in contaminated soil using organic wastes*. International Journal of environmental Science and Technology 10(13),769-778.

Das, R., Tiwary, B. N. 2013.*Isolation of a novel strain of Planomicrobium from diesel contaminated soil of tropical environment*.Journal of Basic Microbiology. 53(2013),723-732.

Dott, W., Feidieker, D., Steiof ,M., Becker, P. M., Kampfer, P. *Comparison of Ex Situ and In situ Techniques for Bioremediation of Hydrocarbon-polluted Soils*. 1995. International Bioremediation and Biodegradation (1995), 301-316.

Duong, T. T. T.2013.*Compost effect on soil properties and plant growth*. The University of Adelaide (PHD Thesis).

Ghanem, A., Orazio, Valeria D., Senesi, N. 2013. *Effects of compost addition on Pyrene removal from soil cultivated with three selected plan species* Clean-Soil, Air, Water 2013,41(12),1222-1228.

Gómez-Sagasti, M.T., Alkorta, I., Becerril, J.M., Epelde, L., Anza, M., Garbisu, C. 2012. *Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation*. Water Air Soil Pollution. 223(12),3249–62.

Gaskin, S.E., Bentham, R.H. 2010.*Rhizoremediation of hydrocarbon contaminated soil using Australian native grasses*. Sci. Total Environ. 408(10), 3683–88.

Gunther, T., Domberger,U., Fritsche, W.1996. *Effects of rye grass on bioremediation of hydrocarbons in soil*. Chemosphere.33(96),203-215.

Halla, J., Sooleb, K., Benthama, R. 2011. *Hydrocarbon Phytoremediation in the Family Fabacea—A Review*.pages 317-332. International Journal of Phytoremediation.Volume 13, Issue 4.

Ibraham, M.M., Alsahli, A.A., El-Gaali G. 2013. *Evaluation of phytoremediation potential of six wild plants for Metals in a site polluted buinduterial wastes: A field study in Riyadh, Saudi Arabia*. Pak.J. Bot., 42(2): 571-576.

Kechavarzi, C., Pettersson, K., Harrison, P. L ., Ritchie, L., Ledin, S. 2007. *Root establishment of Perennial ryegrass (L.Perenne) in diesel contaminated subsurface soil layers*. J Environmental Pollution 145(2007) 68-74.

Komilis, D. P., Vrohidou, Aggeliki- Eleni. K., Voudrias, E. A. 2010. *Kinetics of Aerobic Bioremediation of Diesel –Contaminated Sandy Soil: Effect of Nitrogen Addition*. Journal of Water Air Soil Pollut 208(10),193-208.

Kirk, J. L., Klironomos, J. N., Lee, H., Trevors, J. T. 2005. *The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil*. J of Environmental Pollution 133(2005) 455-465.

Klute, A. 1986. *Methods of Soil Analysis: part1 Physical and Mineralogical Methods*. Second edition. Soil Science Society of America, Maddison, Wisc, USA.

Ling, C.C., Isa, M. H. 2006. *Bioremediation of oil sludge contaminated soil by co-composting with sewage sludge*. Journal of Science& Industrial Research.Vol.65 (2006), 364-369.

Namkoong, W. Hwang, E-y., Park, J-S., Choi, J-Y. 2002. *Bioremediation of diesel- contaminated soil with composting*. J Environmental Pollution (ELSVIER) 119(2002), 23-31.

Palmroth, M. R.T., Pichtel, J., Puhakka, J. A.2002. *Phytoremediation of subarctic soil contaminated with diesel fuel.* J Bioresource Technology. 84(02), 221-228.

Phillips, L. A., Greer, C. W., Farrell, R. E. a., Germida, J.J. 2012. *Plant root exudates impact the hydrocarbon degradation potential of a weathered-hydrocarbon contaminated soil*. Applied Soil Ecology 52 (12) 56– 64.

Pilon-Smits, E. 2005. *Phytoremediation*. Annu Rev Plant Biol 56(05), 15-39.

Racine, C.H. 1994. *Long-term recovery of vegetation on two experimental crude oil spills in interior Alaska black spruce taiga*. Can. J. Bot. 72(94) 1171-1177.

Razmjoo,K., Adavi,Z. 2012. *Assessment of Bermuda grass Cultivars for phytormediation of Petroleum Contaminated Soils*. International Journal of Phytoremediation. 14(12),14-23.

Saadoun, I. M.K., Al-Ghazawi, Z. D. 2005. *Bioremediation of Petroleum Contamination*. Editors: Milton Fingerman,R.Nagaabhushanam, Taylor and Francie.

SAS Institute Inc. 2013.Using JMP 11. Cary, NC: SAS Institute Inc.

Shahsavari, E., Adetuta, E. M., Anderson, P. A., Anderson, A. S. 2013. *Tolerance of selected plant species to petrogenic hydrocarbons and effect of plant rhizosphere on the microbial removal of hydrocarbons in contaminated soil*. Water, Air& Soil Polllution 224:1495 DOI 10.1007/ s 11270-013-1495-3.

Shahsavari, E., Adetuta, E. M., Ball, A. S. 2014. *Phytoremediation and Necrophytoremediation of Petrogenic Hydrocarbon- Contaminated soils*. (http://WWW.researchgate.net/publication/266839143).

Speight, J. G., Arjoon, K. 2012. *Bioremediation of Petroleum and Petroleum Products*.

(http:// www.Cleanglobe. Eu/press 220208.html,2009).

US- EPA Method 1664. February 2010. *Revision B: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry*. February 2010.

United States Salinity Laboratory Staff.1954.*Diagnosis and improvement of saline and alkali soils*.Agricultural Handbook No. 60.USDA. Washington, D.C.20402.