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Assessment of Certain Plant Species degrading Total Petroleum Hydrocarbons in Contaminated Soil

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Abstract
Biological techniques, especially phytoremediation, have long been recognized as cost-effective and environment friendly to eliminate pollutants from soil. This article is based on a study conducted to assess the capability of alfalfa, ryegrass and white clover to remove total petroleum hydrocarbons (TPHs) from soil. The presence of petroleum contamination significantly decreased germination percentage and rate along with biomass of alfalfa and white clover compared to uncontaminated soil. With regards to ryegrass, there was no significant difference in seed germination percentage and biomass, although the presence of petroleum decreased seed germination rate. The results indicated that these plants had effect on TPHs remediation; and removal of TPH from soil was directly related to density levels and time. Therefore, alfalfa and ryegrass in their highest density levels reduced the maximum concentration of TPHs at the end of the experiment by almost 64.41% and 60.36%, respectively, whereas only slight changes were observed in non-vegetated soil.

Keywords
Phytoremediation; Contaminated Soil; Alfalfa; Ryegrass; White Clover

Introduction

Total petroleum hydrocarbons (TPHs), which mainly consist of hydrocarbons, aromatic compounds and paraffin (Akpor *et al*., 2007), present group of persistent organic contaminants widely found in the environment, particularly in the form of saturated TPHs, which can be found in soils and sediments, groundwater and the atmosphere (Bamforth and Singleton, 2005). Pipeline leakage, oil refinery processes, and accidental spills (Jernelov, 2010), as well as coal liquefaction and gasification, volcanic eruptions, forest fires or run-off (Fabbri *et al*., 2003), are among the factors contributing to the presence of these compounds in our environment. Studies have indicated some genotoxic effects of these contaminants for humans and ecosystem (Haeseler *et al*., 1999; Gaoand Zhu, 2004; Oleszczuk and Baran, 2007). These pollutants are being removed or reduced only at a slow pace, while low water solubility and high recalcitrant properties have led to their persistence in soil (Kottler and Alexander, 2001). Although different petroleum treatment techniques, including physical, chemical and biological methods, have been used for this purpose, such methods have not been cost-effective (Henner *et al*., 1997). Among cost-effective techniques, like biological methods which include phytoremediation, plants are used, for example, in clean-up of soils, groundwater or other contaminated circumstances, and natural attenuation by biodegradation and physiochemical mechanisms (Schnoor *et al*., 1995; Joner and Leyval, 2001; McCutcheon, 2004; Watanabe *et al*., 2009). Phytoremediation, or the use of vegetation *in situ* to “clean up” land, has a number of advantages over other approaches, such as: (1) the natural structure and texture of the soil can be retained through this method; (2) the method is based on the energy that can be derived from sunlight; (3) the amount of biomass in the soil can be considerably improved; and (4) it can be carried out quickly (Glick, 2003; Yuan Peng *et al*., 2010). The essential objective of planting in TPH-contaminated soil is the breakdown of TPHs taken up by a combination of mechanisms of plant and soil interactions. Such mechanisms include a rise in soil microbial activity, recovery of physical and chemical properties of contaminated soil and increase in interaction between rhizosphere microbes and the toxic compounds in a contaminated soil (Aprill and Sims, 1990). Some environmental advantages of this approach include reduced wind erosion, less surface water run-off, reinforcement of soil by roots, and increased water infiltration (Harris *et al*., 1996). Effects of plants on petroleum contaminants have been reported enormously in the literature (Banks *et al*., 2003; Sung *et al*., 2003; Liste & and Prutz, 2006; Mueller & and Shann, 2006). Notably, enhanced degradation of pyrene by nine plant species was reported by Liste and Alexander (2000) who found a 74% reduction in planted soil compared to unplanted soil (having less than 40% reduction). Another study indicated that in a 12-week period 46% of crude oil was removed by planting broad bean, whereas the amount of crude oil removed was only 33% in non-vegetated soil (Radwan *et al*., 2000).

Moreover, in addition, a number of different factors may be taken into account in order to maximize reduction of contaminants and to stabilize vegetation cover. To achieve maximum root surface area, particular plants should be chosen very carefully (Aprill and Sims, 1990). It has also been documented that only the plants native to an area in which they are grown can tolerate poor availability of nutrients (Harris *et al*., 1996). Many other studies have indicated that grasses can reduce soil contamination as they have fibrous root system with extensive surface area facilitating microbial colonization (Adam *et al*., 2002). Another strand of research has shown that legumes can be used for phytoremediation as nitrogen often limits mineralization of organic contaminants in soil. Increased level of carbon caused by TPH contaminants stimulates microbial activities, thereby, creating an imbalance in the soil C-N ratio, which, in turn, can immobilize nitrogen by the microbial biomass. Furthermore, it is better to cover petroleum contaminated soil using legumes that can fix atmospheric nitrogen (Gudin and Syratt, 1975).

The Tehran oil refinery is one of the most crucial crude oil refineries in Iran, where oily waste of refinery has led to environmental pollution and changes in some parts of the ecosystems. While different approaches have been used to remediate these pollutants, phytoremediation can be proposed as the suitable method to reduce petroleum hydrocarbons around the Tehran oil refinery. The article embodies the experiment done to investigate the efficiency of phytoremediation process in the contaminated soil by comparing the ability of three different plants (one grass and two legumes) grown in petroleum contaminated soils. Indicators such as germination percentage, germination rate and biomass of these plants, as well as their ability to degrade contaminants in soil, have been selected to ascertain the most successful plant species. The results of this experiment show that the phytoremediation is a viable measure to halt the petroleum-based pollutants in the soil.

Materials and Methods

Soil Preparation

To conduct the experiment, bulk samples of petroleum-contaminated and uncontaminated soils were collected, respectively, from waste oil landfill in Tehran oil refinery ($35^0$30' –35^0 31'S, 51^0 24' – 51^0 27' E) and from an area located far from the refinery. To homogenize and create a uniform mixture, the soils were mixed, air dried, and then passed through a 4-mm sieve**.** The sampled soils were labelled as C0 (uncontaminated soil) and C1(contaminated soil).

Soil Characteristics

After sieving using a 2 mm sieve, some physical and chemical properties of contaminated and uncontaminated soil samples were measured (Table1). Soil pH was measured by a digital pH-meter (Thomas, 1996), electrical conductivity (EC) by an EC-meter (Rhoades, 1996), organic matter percentage by the Walkley-Black method (Black *et al*. 1965), the CaCO3, Ca and Mg percentage by titration method (Black *et al*., 1965), total N by the micro-Kjeldahl method (Bremner, 1996), available-P content by Olsen method (Houba *et al*., 1989) and available-K by Ammonium acetate method (Hemek and Spark, 1996).

TPHs Analysis

To measure concentration of TPHs, using the gravimetrical method (Peng *et al*., 2009), firstly, 5 g of dry contaminated soil was weighed and placed in a 40 ml glass centrifuge tube. Secondly, 25 ml solvent dichloromethane was added and then the samples were ultrasonically extracted for 1hour. Thirdly, the samples were centrifuged for 10 min under 3000 rpm. Next, the supernatant was transferred to an Erlenmeyer flask to evaporate volatile dichloromethane. After evaporation of the solvent under 65˚C, vis-a-vis the initial weight of flask, TPHs concentrations was obtained by weighing it. The initial concentration of TPHs in contaminated soil is shown in Table1.

Initial Testing for Germination and Growth

Three plants namely alfalfa (*Medicago sativa* L.), ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) were tested. The soil samples were added to plastic pots of 22 cm in diameter and 16 cm in height, and seeds of the plants were sown in three density levels (the minimum density level: I, the average density level: II, the maximum density level: III) and 1-2 cm deep in the pots. To determine density levels of plants, the minimum and maximum consumption of seeds per hectare was considered along with the values converted to the surface areas of the pots. The plants were irrigated from the top during the germination period so that soil moisture was kept near field capacity and the number of germinated seeds in each pot was recorded as a germination percentage during trial period. Germination rate was derived by taking the germination count during 14 days and estimated according to the Maguire method (Maguire, 1962).

Phytoremediation Experiment

To investigate the stability, plants were kept for 150 days in soils. During the trial, the soil was sampled on interval of days 30, 60, 90, 120 and 150 to determine the concentration of TPHs in the samples by utilizing the gravimetrical method in 3 replications. At the end of the period, all plants (shoot and root) were harvested then washed by deionized water and dried in an oven at 80˚C for 24 hours, and finally weighed.

Data Analysis

This experiment was conducted as a factorial experiment with randomized complete design in triplicate. Results were examined by analysis of variance using Statistical Analysis Software (SAS) and MSTAT-C and mean comparison via Least Significant Difference (LSD) method. The germination percentage and rate at the level of $P<0.01$, dry matter yield at the level of $P<0.05$ and phytoremediation experiment at the level of $P<0.01$ were carried out.

Table 1: Selected physical and chemical properties of soil samples

Characteristic	Contaminated soil (C1)	Uncontaminated soil (C0)
Texture	Clay-loam	Clay-loam
PH	7.34	7.69
ECe (ms)	2.33	1.484
$CaCO_3$ (%)	16.5	25
OM (%)	4.09	0.76
Total N (%)	0.19	0.036
Available-P (mg/kg)	17.24	16.8
Available-K (mg/kg)	344.3	154.75
Ca (meq/lit)	26	12.5
Mg (meq/lit)	9.6	17
Initial of TPHs (g/kg)	14.8	0

Table2. Average percentage of seed germination of plants with different density levels

Plant species	Contaminated soil (C1)	Uncontaminated soil (C0)
Alfalfa II	75.83^{b}	96.67^{a}
Alfalfa III	77.97^{b}	97.74^{a}
White clover II	67.77^{b}	98.89^{a}
White clover III	63.12^{b}	98.58^{a}
Ryegrass II	93.66^{a}	97.35^{a}
Ryegrass III	98.37^{a}	99.19^{a}

(Similar letters based on LSD test showed no significant difference ($P>0.01$))

Table3. Average germination rate of plants with different density levels

Plant species	Contaminated soil (C1)	Uncontaminated soil (C0)
Alfalfa II	5.94[b]	7.75[a]
Alfalfa III	8.95[b]	11.8[a]
White clover II	3.11[b]	5.14[a]
White clover III	4.61[b]	7.71[a]
Ryegrass II	7.04[b]	8.04[a]
Ryegrass III	9.14[b]	11.13[a]

(Similar letters based on LSD test showed no significant difference (P>0.01)

Results and Discussion

Germination Percentage and Rate

Percentage of germination was measured by taking into account the density levels II and III in the three plant populations. The effect of petroleum contamination on germination percentage of plants is shown in Table 2.

There were no significant differences (P >0.01) in terms of seed germination percentage of ryegrass between C1 and C0 during the experimentation period. While seed germination of alfalfa and white clover was significantly (P < 0.01) decreased in C1 compared to the C0 (Table 2). At the end of 14 days, there were over 20% and 33% decreases in germination of alfalfa and white clover seeds, respectively, in both density levels in C1 treatment as compared to C0 (Figure 1). The ability of the soil to provide nutrients and water required for seed germination and growth of plants is indirectly influenced by the presence of petroleum hydrocarbons (Reilly *et al*., 1996).

Considerable variation was found in seed germination rate in the plants studied in this experiment (Table 3). The presence of petroleum contamination in C1 soils significantly (P<0.01) decreased germination rate of plants: 40% for white clover, 23% for alfalfa, and 17% for ryegrass compared to C0 specimens. Seed germination of ryegrass and white clover started 1 day late in C1 treatment compared to C0 (Figure 2).

Evidently, the presence of petroleum contamination in soil creates a film of oil around the seed, thereby preventing and reducing water and oxygen transfer to the seed. As a result of this physical hindrance, a delay in seed emergence was noticed. It could be an additional factor in the overall inhibitory effect of petroleum hydrocarbons on germination (Adam *et al*., 2002). Similar results were obtained by different researchers in seed germination percentage and rate (Crafts and Reiber, 1948; Schwendiger, 1968; Udo and Fayemi, 1975; Warner *et al*., 1984; Longpre *et al*., 1999; Smith *et al*., 2006). For instance, Epuri and Sorensen (1997) found that tall fescue (*Festuca arundinacea*) had the highest percentage of germination among four different grasses they had grown in contaminated soil. Plants have different responses to different concentrations of pollution due to various physiological properties. Adam and Duncan (2002) reported that diesel fuel plays an influential role in delaying seed emergence and reducing the percentage of germination; however, seed germination response varied greatly with plant species: some were notably tolerant, while others were intolerant. Furthermore, Liste and Prutz (2006) found no reduction in ryegrass, and 26% reduction in germination percentage of alfalfa when planted in petroleum contaminated soil. According to the findings of Dibble and Bartha (1979), germination of wheat and soybean experienced a delay in kerosene-contaminated soil (34%), but the germination percentage of contaminated soil reached the same level as that of uncontaminated soils after 10 days; this delay of germination in contaminated soil was due to reduced availability of oxygen and competition between plants and microorganisms for using oxygen.

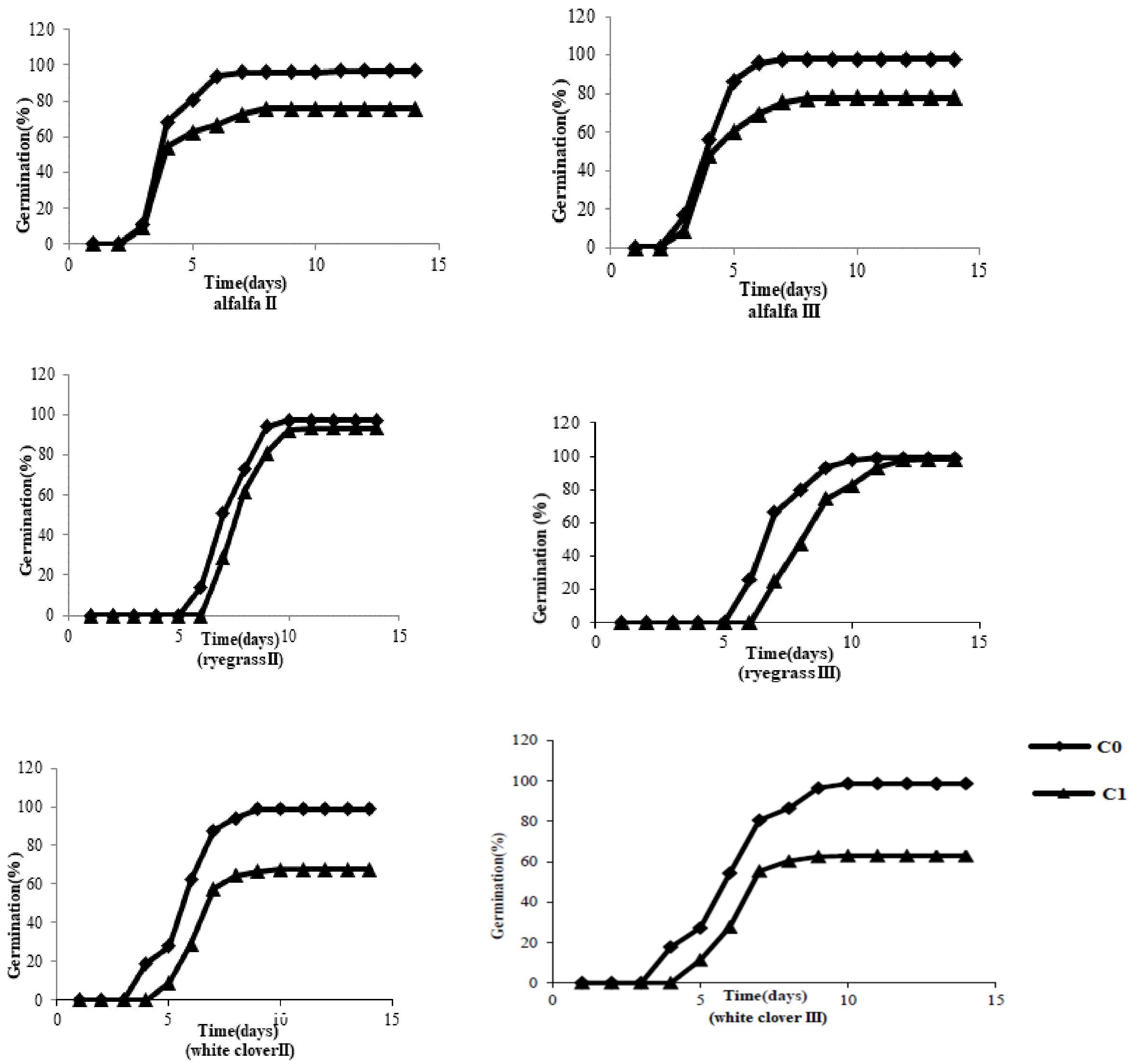

Figure 1. The changes in germination percentage in A- alfalfa B- ryegrass C- white clover respectively. (The left side figures are II density and the right side figures are III density)

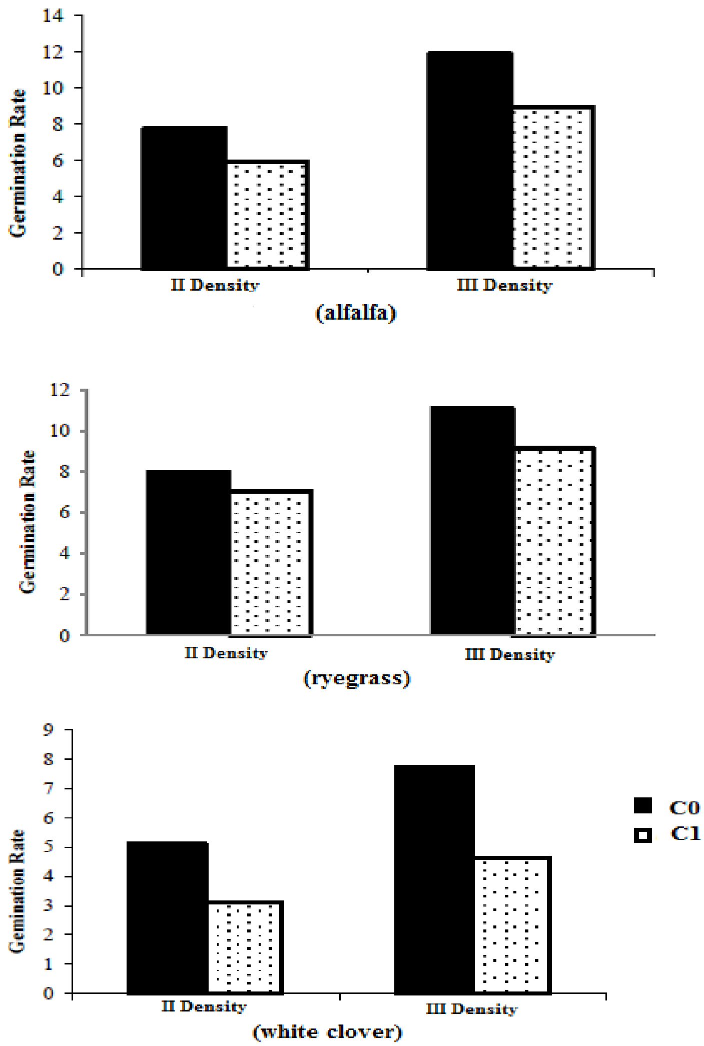

Figure 2. Changes in germination rate during time in different density levels (II and III) in Plants

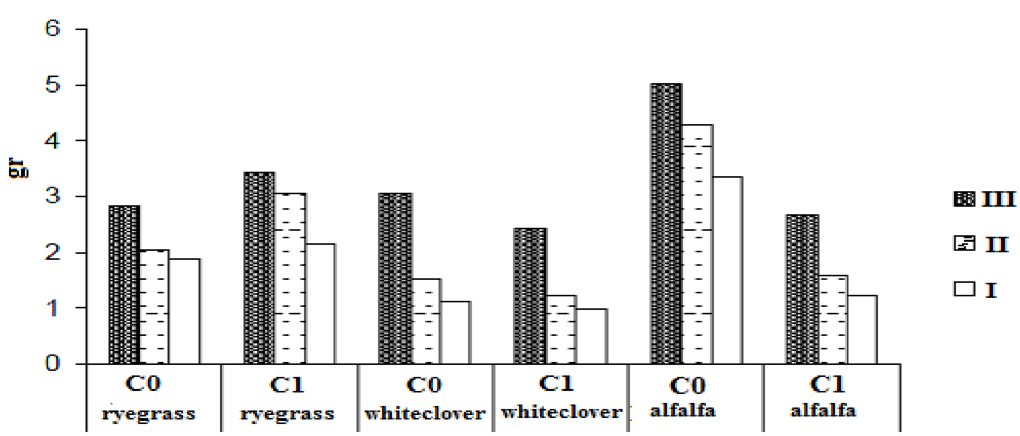

Figure 3: Dry weight of root plants in different density in C1 and C0 soils

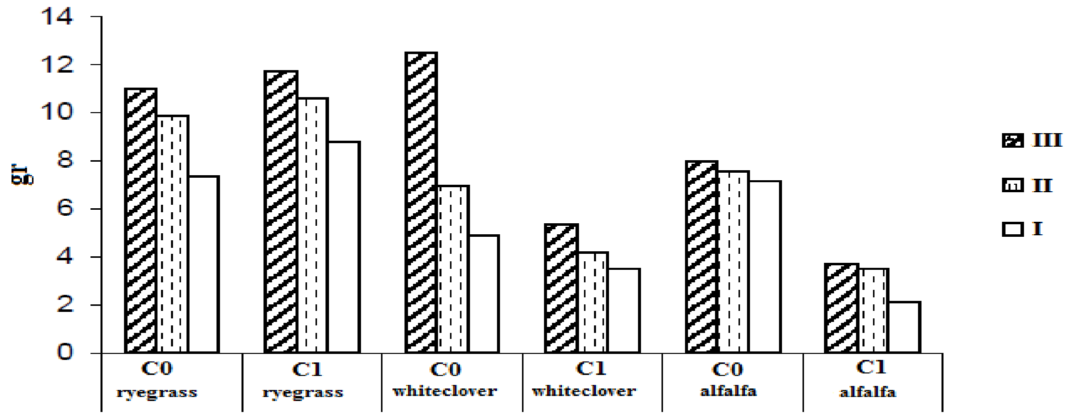

Figure 4: Dry weight of shoot plants in different density levels in C1 and C0 soils

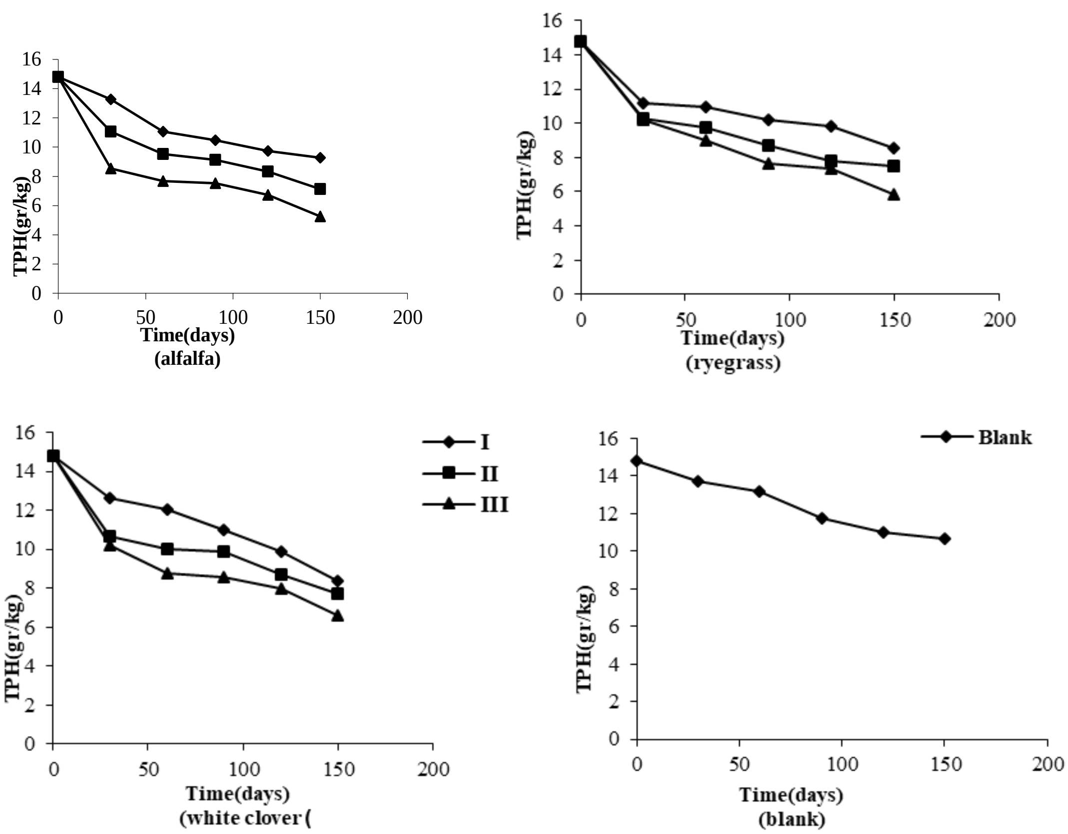

Figure 5. The changes in concentration of TPH in soil over the time in different density levels of plants

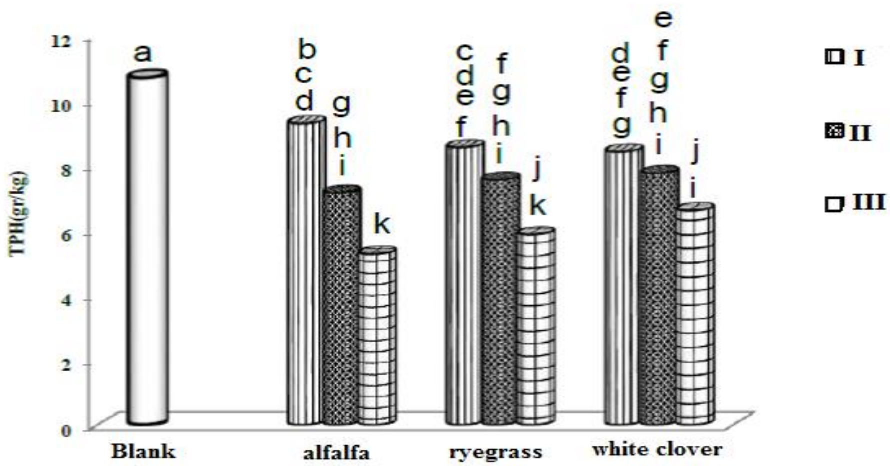

Figure 6. The changes in concentration of TPH in vegetated and non-vegetated soils at the end of 150 days (Similar letters based on LSD test showed no significant difference (P>0.01))

Total Dry Matter Yield

Dry matter yield of plants in three different density levels after 150 days experimentation period is shown in Figure 3 and 4. There was a significant ($P < 0.05$) reduction in the shoot biomass of alfalfa and white clover in contamination treatments (approximately 50%). However, there was no significant difference ($P > 0.05$) between C1 and C0 in terms of the root biomass of white clover during the experimentation period. A slight decrease (almost 20%) in root weight at all density levels was found in C1 compared to C0. On the other hand, the root biomass of alfalfa significantly ($P < 0.05$) decreased in C1 which accounted for nearly 60% compared to uncontaminated soil at all three density levels. Moreover, there was no significant difference ($P > 0.05$) in the ryegrass shoot and root biomass in the presence of TPHs in contamination treatments. Nevertheless, there was an increase in the shoot and root weight, 6% and 20% respectively, in C1 as compared to C0. It seems that the movements of roots have a serious problem owing to toxicity of petroleum contamination over this period and this has a critical effect on the root and shoot of leguminous plants. On the other hand, petroleum compounds have indirect effect by stimulating the growth of bacteria which can reduce plant growth by consuming oxygen and nutrients. However, ryegrass was able to tolerate this pollutant and even it attained its optimal growth. Recent studies illustrated reduced or unchanged growth of plants. Some studies reported that poor germination of seeds is related to the subsequent poor growth in TPHs soils but others did not prove this (Chaˆıneau *et al*., 1997; Wiltse *et al*., 1998; Carr, 1999).

This experiment has shown a correspondence between good germination and subsequent growth. Ryegrass seedlings were tolerant to TPHs; no significant reduction was found in the dry matter yield of this genotype in contaminated soils, and growth of this plant in C1 was found to be equal or slightly greater than the values seen in C0. In contrast, white clover and alfalfa had low seed germination percentage and germination rate in contaminated soils and a marked reduction was found in their growth and dry matter yield as compared to the control. Joner and Leyval (2001) reported that the growth of white clover reduced significantly in soils contaminated by Anthracene, Dibenz [a,h] and kerosene. Additionally, Fan *et al*., (2008) observed a reduction in root and shoot biomass of alfalfa in different concentrations of pyrene in contaminated soil, but ryegrass was not affected by this pollutant.

Phytoremediation Experiment

The effect of plants on petroleum contamination in soils is shown in Figure 5. Plant species and density levels had significant ($P < 0.01$) effect on reduction of TPHs. In other words, this experiment showed that all three plant species were able to tolerate contaminated soil and reduce the concentration of TPHs. On the other hand, there was a significant difference ($P < 0.01$) in the time required to lower the concentration of TPHs, and also the highest reduction was related to the first 30 days of experimentation period and this rate decreased in contaminated soil over time (Figure 5). Purves *et al*. (1998) reported that organic pollutants persisting in soil for a long time will be out of the reach of plants. So, the amount of absorption as well as decomposition was reduced by plants with the passage of time. Alexander (1999) observed that biological availability of contaminants could be lessened in different percentages of petroleum in soil over the time; this was done through absorption processes by solid particles of soil, or bond with organic matters, or fixation by soil microsites that led to disruption in access by plants. Tam and Wong (2008) reported that the highest decrease in concentration of Polycyclic aromatic hydrocarbons (PAH_s)was observed in the first 30 days, reduced after 90 days, and reached a level similar to the one found in 154 days at the end of the trial.

The results of this experiment showed that over time different density levels of plants led to significant reductions ($P < 0.01$) in concentrations of TPHs in vegetated soils compared to non-vegetated soil (blank). However, density I of white clover was the most effective in comparison with the others, but in higher levels of densities of plants, alfalfa had the greatest effect on degradation. Therefore, increase in density levels of plants, in other words, with rising the number of plants in a unit area by occupying a vast surface and infiltrating into soil leading to decrease pollutants in per unit. Density I, density II and density III of alfalfa degraded TPHs concentration by 37.39%, 51.80% and 64.41%, respectively, in 150 days. In ryegrass, treatment resulted in removal of 42.34% (density I), 49.10% (density II) and 60.36% (density III) of TPHs in 150 days. Finally, densities I, II and III of white clover removed 43.24%, 47.75% and 55.40%, respectively, of TPHs at the end of the experiment; while in the non-vegetated treatment, TPHs concentration decreased more slowly compared to other soil vegetation in 150 days (about 27.93%). Hence, the largest reduction in TPHs was observed in alfalfa and ryegrass especially in their highest density levels from an initial 14.8 g kg^{-1} to 5.26 g kg^{-1} and 5.86 g kg^{-1} respectively (Figure 6). In general, it seems that the vegetation can enhance the degradation of organic pollutants in soil by increasing biological availability of hydrocarbons, the root secretions and soil improvement. Many studies have shown that different plant species help degrade toxic organic contaminants (Chaˆıneau *et al*., 2000; Xu *et al*., 2006; Rezek *et al*., 2008). For instance, Wilts *et al*. (1998) found that different species of alfalfa had positive effect on removing pollutants. The plant reduced 33-56% of crude oil contamination compared to non-vegetated treatment. Nedunuri *et al*. (2000) reported that ryegrass was the more effective than sorghum for TPHs remediation. Furthermore, Gao and Zhu (2004) observed that degradation of phenanthrene and pyrene increased in soils planted with various plant species including ryegrass. The presence of the plants in contaminated soils significantly contributed to the degradation of pollutants.

Conclusion

This study showed that the presence of specific plants accelerates removal of petroleum from the soil. However, the remediation depends on the density levels and time factors. On the one hand, the considerable decline has occurred in the highest density levels of alfalfa and ryegrass. On the other hand, the maximum reduction happened in the first 30 days of experimentation period and this rate decreased in contaminated soil over the time. All three plant species tested tolerated the TPHs concentration in contaminated soil. However, Treatment of petroleum contaminated soil with ryegrass has proved to be more effective method for TPHs remediation owing to its special root systems with extensive surface that can enhance the contact more with the pollutant compounds. Moreover, its ability in tolerating toxicity of petroleum by the highest germination percentage and biomass in comparison with other plants, while no significant difference in terms of degrading TPHs degradation when compared to alfalfa has indicated that we select ryegrass as a candidate species for phytoremediation of petroleum contaminated soil.

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References

Adam, G. and Duncan, H. (2002). Influence of diesel fuel on seed germination. *Environmental Pollution*, 120(2): 363-370.

Adam, G., Gamoh, K., Morris, D.G. and Duncan, H. (2002). Effect of alcohol addition on the movement of petroleum hydrocarbon fuels in soil. *Science of the Total Environment*, 286(1-3):15-25.

Akpor, O.B., Igbinosa, O.E. and Igbinosa, O.O. (2007). Studies on the effect of petroleum hydrocarbon on the microbial and physico-chemicals characteristics of soil. *African Journal of Biotechnology*, 6(16).

Alexander, M. (1999). *Biodegradation and bioremediation*. Texas, USA: Gulf Professional Publishing.

Aprill, W. and Sims, R.C. (1990). Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere*, 20(1-2): 253-265.

Bamforth, S.M. and Singleton, I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology & Biotechnology*, 80(7): 723-736.

Banks, M.K., Schwab, P., Liu, B., Kulakow, P.A., Smith, J.S. and Kim, R. (2003). The effect of plants on the degradation and toxicity of petroleum contaminants in soil: a field assessment. In *Phytoremediation* (pp. 75-96). Berlin/Heidelberg: Springer.

Black, C.A., Evans, D.D., White, J.L., Ensminger, L.E. and Clark, F.E. (1965). Methods of soil analysis. Part 2. American Society of Agronomy, Madison, Wisconsin, USA, Agronomy Series No. 9, 801.

Bremner, J.M., Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T. and Sumner, M.E. (1996). Nitrogen-total. *Methods of soil analysis. Part 3-chemical methods*, pp.1085-1121.

Carr, R.H. (1999). Vegetative growth in soils containing crude petroleum. *Soil Science*, *8*(1): 67-68.

Chaineau, C.H., Morel, J.L. and Oudot, J. (1997). Phytotoxicity and plant uptake of fuel oil hydrocarbons. *Journal of Environmental Quality*, 26(6): 1478-1483.

Chaineau, C.H., Morel, J.L. and Oudot, J. (2000). Biodegradation of fuel oil hydrocarbons in the rhizosphere of maize. *Journal of environmental quality*, 29(2): 569-578.

Crafts, A. and Reiber, H. (1948). Herbicidal properties of oils. *Hilgardia*, 18(2): 77-156.

Dibble, J.T. and Bartha, R. (1979). Rehabilitation of oil-inundated agricultural land: a case history. *Soil Science*, 128(1): 56-60.

Epuri, V. and Sorensen, D. L. (1997). Chapter 15 Benzo(a)pyrene and hexachlorobiphenyl contaminated soil: phytoremediation potential. In E.L. Kruger, T.A. Anderson and J. R. Coats (Eds.), *Phytoremediation of Soil and Water Contaminants*. American Chemical Society: Washington, D.C., ACS Symposium Series 664, pp.200-222.

Fabbri, D., Vassura, I., Sun, C.G., Snape, C.E., McRae, C. and Fallick, A.E. (2003). Source apportionment of polycyclic aromatic hydrocarbons in a coastal lagoon by molecular and isotopic characterization. *Marine chemistry*, 84(1-2): 123-135.

Fan, S., Li, P., Gong, Z., Ren, W. and He, N. (2008). Promotion of pyrene degradation in rhizosphere of alfalfa (Medicago sativa L.). *Chemosphere*, 71(8): 1593-1598.

Gao, Y. and Zhu, L. (2004). Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. *Chemosphere*, 55(9): 1169-1178.

Glick, B.R. (2003). Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnology advances*, 21(5): 383-393.

Gudin, C. and Syratt, W.J. (1975). Biological aspects of land rehabilitation following hydrocarbon contamination. *Environmental Pollution*, 8(2): 107-112.

Haeseler, F., Blanchet, D., Druelle, V., Werner, P. and Vandecasteele, J.P. (1999). Ecotoxicological assessment of soils of former manufactured gas plant sites: bioremediation potential and pollutant mobility. *Environmental Science & Technology*, 33(24): 4379-4384.

Harris, J.A., Birch, P. and Palmer, J.P. (1996). *Land restoration and reclamation: principles and practice*. Boston, USA: Addison Wesley Longman Ltd.

Hemke, P.H. and Spark, D.L. (1996). Potassium. 551-574.In Sparks, D.L. *et al.* (1996), *Method of soil analysis.* Madison, Wisconsin, USA: Soil Science Society of America Inc. & American Society of Agronomy, Inc.

Henner, P., Schiavon, M., Morel, J.L. and Lichtfouse, E. (1997). Polycyclic aromatic hydrocarbon (PAH) occurrence and remediation methods. *Analusis*, 25(9-10): M56-M59.

Houba, V.J.G., Van der Lee, J.J., Novozamsky, I. and Walinga, I. (1989). Soil and plant analysis a series of syllabi, Part 5: Soil Analysis Procedures. Wageningen Agriculture University, Department of Soil Science and Plant Nutrition, Dreijenplein, 10.

Jernelöv, A. (2010). How to defend against future oil spills. *Nature*, 466(7303), p.182.

Joner, E.J. and Leyval, C. (2001). Influence of arbuscular mycorrhiza on clover and ryegrass grown together in a soil spiked with polycyclic aromatic hydrocarbons. *Mycorrhiza*, 10(4): 155-159.

Kottler, B.D. and Alexander, M. (2001). Relationship of properties of polycyclic aromatic hydrocarbons to sequestration in soil. *Environmental pollution*, 113(3): 293-298.

Liste, H.H. and Alexander, M. (2000). Plant-promoted pyrene degradation in soil. *Chemosphere*, 40(1): 7-10.

Liste, H.H. and Prutz, I. (2006). Plant performance, dioxygenase-expressing rhizosphere bacteria, and biodegradation of weathered hydrocarbons in contaminated soil. *Chemosphere*, 62(9): 1411-1420.

Longpre, D., Jaouich, A., Jarry, V., Venosa, A.D., Lee, K. and Suidan, M.T. (1999). The response of *Scirpus pungens* to crude oil contaminated sediments. Phytoremediation Technical Seminar, Calgary (Canada) 31, pp.137-157.

Maguire, J.D. (1962). Speed of Germination—Aid in Selection and Evaluation for Seedling Emergence and Vigor 1. *Crop science*, 2(2): 176-177.

McCutcheon, S.C. and Schnoor, J.L. (2004). *Phytoremediation: transformation and control of contaminants* (Vol. 121). Hoboken, New Jersey, USA: John Wiley & Sons.

Mueller, K.E. and Shann, J.R. (2006). PAH dissipation in spiked soil: Impacts of bioavailability, microbial activity, and trees. *Chemosphere*, 64(6): 1006-1014.

Nedunuri, K.V., Govindaraju, R.S., Banks, M.K., Schwab, A.P. and Chen, Z. (2000). Evaluation of phytoremediation for field-scale degradation of total petroleum hydrocarbons. *Journal of Environmental Engineering*, 126(6): 483-490.

Oleszczuk, P. and Baran, S. (2007). Polyaromatic hydrocarbons in rhizosphere soil of different plants: effect of soil properties, plant species, and intensity of anthropogenic pressure. *Communications in soil science and plant analysis*, 38(1-2): 171-188.

Peng, S., Zhou, Q., Cai, Z. and Zhang, Z. (2009). Phytoremediation of petroleum contaminated soils by Mirabilis Jalapa L. in a greenhouse plot experiment. *Journal of hazardous materials*, 168(2-3): 1490-1496.

Purves, W. K., Orians, G. H., Heller, H. C. and Sadava, D. (1998). *Life: The Science of Biology*. Salt Lake City, USA: Sinauer Associates Inc. & W.H. Freeman and Company:

Radwan, S.S., Al-Awadhi, H. and El-Nemr, I.M. (2000). Cropping as a phytoremediation practice for oily desert soil with reference to crop safety as food. *International journal of phytoremediation*, 2(4): 383-396.

Reilley, K.A., Banks, M.K. and Schwab, A.P. (1996). Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. *Journal of Environmental Quality*, 25(2): 212-219.

Rezek, J., Mackova, M., Zadrazil, F. and Macek, T. (2008). The effect of ryegrass (Lolium perenne) on decrease of PAH content in long term contaminated soil. *Chemosphere*, 70(9): 1603-1608.

Rhoades, J.D. (1996). Salinity: Electrical conductivity and total dissolved solids. *Methods of Soil Analysis Part 3—Chemical Methods* (methodsofsoilan3), pp.417-435.

Schnoor, J.L., Light, L.A., McCutcheon, S.C., Wolfe, N.L. and Carreia, L.H. (1995). Phytoremediation of organic and nutrient contaminants. *Environmental Science & Technology*, 29(7): 318A-323A.

Schwendinger, R.B. (1968). Reclamation of soil contaminated with oil. *J. Inst. Petrol*, 54,: 182-197.

Smith, M.J., Flowers, T.H., Duncan, H.J. and Alder, J. (2006). Effects of polycyclic aromatic hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil and soil with aged PAHs residues. *Environmental pollution*, 141(3): 519-525.

Sung, K., Munster, C.L., Rhykerd, R., Drew, M.C. and Corapcioglu, M.Y. (2003). The use of vegetation to remediate soil freshly contaminated by recalcitrant contaminants. *Water Research*, 37(10): 2408-2418.

Tam, N.F.Y. and Wong, Y.S. (2008). Effectiveness of bacterial inoculum and mangrove plants on remediation of sediment contaminated with polycyclic aromatic hydrocarbons. *Marine Pollution Bulletin*, 57(6-12): 716-726.

Thomas, G.W. (1996). Soil pH and soil acidity. *Methods of Soil Analysis Part 3—Chemical Methods* (methodsofsoilan3), pp.475-490.

Udo, E.J. and Fayemi, A.A.A. (1975). The Effect of Oil Pollution of Soil on Germination, Growth and Nutrient Uptake of Corn 1. *Journal of Environmental Quality*, 4(4): 537-540.

Warner, M.L., Sauer, R.H. and Carlile, D.W. (1984). Barley growth in coal liquid and diesel liquid fuels from coal and oil: a comparison of potential toxic effects on barley. *Water, Air, and Soil Pollution*, 22(1): 47-55.

Watanabe, T., Murata, Y. and Osaki, M. (2009). Amaranthus Tricolor Has the Potential for Phytoremediation of Cadmium-Contaminated Soils. *Communications in soil science and plant analysis*, 40(19-20): 3158-3169.

Wiltse, C.C., Rooney, W.L., Chen, Z., Schwab, A.P. and Banks, M.K. (1998). Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. *Journal of Environmental Quality*, 27(1): 169-173.

Xu, S.Y., Chen, Y.X., Wu, W.X., Wang, K.X., Lin, Q. and Liang, X.Q. (2006). Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation. *Science of the Total Environment*, 363(1-3): 206-215.

Yuan Peng, W., QingBiao, L., Qi, L., JiYan, S. and YingXu, C. (2010). Differences in the Rhizosphere Microbial Activity and Community Composition of *Commelina communis* along a Copper Contamination Gradient. *Communications in soil science and plant analysis*, 41(17): 2046-2056.