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Effect of oil pollution on underground organs of *Frankenia thymifolia* and *Poa bulbosa Turf* in drought stress conditions

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Abstract

Petroleum contamination of soil is a serious problem and of the most important pollutants of the soil throughout the world and country especially sides of oil refinery like Khuzestan province of Iran. The soil contamination could damage the organisms of the soil, including plants and microorganisms. Some plants and microorganisms are able to degrade the oil in the soil. Two types of plants including legumes and grasses have higher potential to be used for phytoremediation. Since grasses used in landscaping to cultivate these plants could achieve two aims: first clean the soil second have a green space. In the present study, a green house experiment was performed on *Frankenia Thymifolia* and *Poa Bulbosa* root yield to introduce these plants to spread the green space on contaminated and hot region in a factorial experiment with a randomized complete block design and eight replicates per treatment. Treatments have done on four levels of different rates of crude oil (0, 1%, 2% and 3% w/w) and four levels of Stress (No stress = FC, $1/4$ stress = 25% FC, $1/2$ stress = 50% FC and $3/4$ stress= 75% FC). Treatments have effect on root biomass and dry mass that significant at ($p<0.01$). The efficiency of root was in level 3% oil pollution that increasing traits than the non pollution in *Frankenia Thymifolia* and *Poa Bulbosa* for about 74.6%, 81.1%, 20.2 % and 15.1% respectively. But not similar results indicate from stress treatments. Interactive effect of crude oil and stress in these plants root yield was significant at ($p < 0.01$). The most wet and dry weight made from treatment of no stress and 3% Oil pollution. With due attention to these results cultivate of *Frankenia Thymifolia* In addition to development of green space in pollution area with oil was so effective but not recommended in hot places. So planting of *Poa Bulbosa* In addition to development of green space in saturate and erosion soil were recommended.

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Introduction

Organic pollutants like petroleum hydrocarbons have had significant share of soil pollution particularly in the last century. Oil products have been disposed of in the environment to significant risks to human through different exposure pathways (Denys *et al.*, 2006). Petroleum hydrocarbons are of the most important organic pollutants of soil in many parts of Iran especially near oil refineries and spill sites (Shahriari *et al.*, 2006). Total petroleum hydrocarbons (T PHs) are one of the most common groups of persistent organic contaminants in the environment and are known to be toxic to many organisms. There are many sources of TPH contaminated in soils including petroleum extract ion, transportation, re fining and consumption (Mac Nicol *et al.*, 1995). The soil quality assessment can be improved by including common hazardous compounds as polycyclic aromatic hydrocarbons (PAHs) and aromatic volatile hydrocarbons like benzene, toluene, Ethylbenzene and xylenes (BTEX) (Pinedo *et al.*, 2013). The toxic effects of hydrocarbon contamination were greater in the sandy soil. Hydrocarbons inhibited microbial biomass, the greatest negative effect being observed in the gasoline-polluted sandy soil (Labud *et al.*, 2007). The growth and reproduction of certain members of the phyla Firmicutes, Actinobacteria and Cyanobacteria were clearly decreased by oil pollution. Oil pollution tended to simplify the functions present in the microorganism communities (Tian *et al.*, 2014). Some of the isolated bacteria, such as members of Rhodococcus genus, displayed a wide potential for hydrocarbon biodegradation and persistence in the studied environment (Peressutti *et al.*, 2003).The development of methods to remediate soils contaminated with toxic pollutants and other organic residues has been an area of intense research interest for several decades (Aprill and Sims 1990; Cunningham *et al.*, 1995). Phytoremediation is an emerging green technology that can be a promising solution to remediate hydrocarbon-polluted soils, not only in developed countries but also in developing countries like Iran, in which uncontrolled disposal of oil industry wastes has polluted soil resources over the past decades. Many plant species are sensitive to petroleum contaminants (Huang *et al.*, 2004). Bioremediation of crude oil contaminated soil is an effective process to clean petroleum contaminant from the environment the combine use of crude oil degrading bacteria along with nutrient supplements could revive crude oil contaminated soil effectively in large scale (Roy *et al.*, 2014). Seed germination, percentage of seedling emergence, number of root nodules/plant, Leghaemoglobin content in root nodules, total soluble sugars, total soluble proteins, free amino acids, total chlorophyll and carotenoids and nucleic acids of the leaves of Vigna mungo (L.) Hepper grown in the polluted soil decreased significantly due to persistence of hydrocarbon (Ilangovan and Vivekanandan, 1992).

The plants were collected on oil contaminated sites in the savannahs of eastern Venezuela and subsequently selected on the basis of characteristics like root morphology, propagation characteristics (seed production, germination rate) and seedling establishment in contaminated soil (Merkletal, 2004). The overall advantage of the chosen grass species is the extensive, widely branched root system providing a large root surface for the growth of microbial populations. Legumes are considered to be especially promising because of their nitrogen independence which is of significant in oil-contaminated soils characterized by a high C/N ratio (Frick *et al.*, 1999). Furthermore, the selected legumes have deep-reaching roots that are capable of penetrating highly compacted soils and Asphaltene soil layers.

Materials and methods

This experiment conducted during the 2012 in Islamic Azad University of Karaj Branch Research farm. Treatments were consisting of different oil pollution levels. (0, 1%, 2% and 3% w/w from crude oil) and four levels of Stress (No stress = FC, $1/4$ stress = 25% FC, $1/2$ stress = 50% FC and $3/4$ stress = 75% FC). And two types of plant (*Frankenia Thymifolia* and *Poa Bulbosa Turf*), a factorial experiment with a randomized complete block design per treatment in eight repeats. The experiment was conducted in a tube with 8cm Diameter and 70 cm Height.

Soil pollution with different levels of crude oil and mix it with sand and manure then abandon for a month. So tube filled with them. Then cutting *Frankenia Thymifolia* and *Poa Bulbosa* seeds were planted. At first irrigation every day until our plants absolutely fixed that twenty days long. Then *Poa Bulbosa* was cut from 3 cm distance from soil surface. For the reason that *Frankenia Thymifolia* have a low growth cutting not accomplish. After these stages exert Stress treatments started. All process taken about 9 weeks then parameters such as (Root weight, root dry mass and root length) were measured. To study the effect of treatments on root yield data results analysis with SPSS and SAS Softwares. And mean comparison done with Duncan test.

Results and discussion

Effect of treatments on Frankenia Thymifolia root wet and dry mass

Result showed that root wet on 3% oil pollution increased to 74.6 than no oil pollution that significant at ($p < 0.01$) table (1). The other hand the driest mass was depended on 3% oil pollution that increased it 81.8% than no polluted (Table 2 and Figure 1). The most wet and dry mass was in Fc conditions (Table 3, Figure 2). Interactive effect of crude oil and stress was significant at ($p < 0.01$).

Table 1. Variance analysis of measurement factors in *Frankenia Thymifolia*.

		Sum of squares *(Frankenia Thymifolia)*	
Source	df	Root weight	Root dry mass
Oil pollution	3	1.553**	0.930**
Stress	3	0.385**	0.482**
Oil pollution*Stress	9	0.127**	0.072*
Error		0.018	0.034

*, ** Show the ($p < 0.05$, $p < 0.01$).

Table 2. Mean comparison of oil pollution application on *Frankenia Thymifolia* root yield.

Treatment	Root wet weight (Per / Plant)	Root dry mass (Per / Plant)
Oil (0% w/w)	0.63 [b]	0.37
Oil (1% w/w)	0.66 [b]	0.28
Oil (2% w/w)	0.69 [b]	0.39
Oil (3% w/w)	1.1 [a]	0.67

*Values within the same column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Table 3. Mean comparison of stress application on *Frankenia Thymifolia* root yield.

Treatment	Root wet weight (Per / Plant)	Root dry mass (Per / Plant)
FC	0.93 [a]	0.61 [a]
FC (75%)	0.77 [b]	0.35 [b]
FC (50%)	0.69 [c]	0.39 [b]
FC (25%)	0.69 [c]	0.36 [b]

*Values within the same column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

The most wet and dry weight made from treatment of no stress and 3% oil pollution that showed 116.9 % than the no stress and no oil pollution. The interactive effect of crude oil and stress on root dry mass significant at ($p < 0.05$) that shown the 136.5% increased than the no stress and no oil pollution. Effect of other treatments is shown in figures 3 and 4. Chaineau *et al.*, (1997) found a growth rate reduction of beans and wheat by more than 80%.

Table 4. Variance analysis of measurement factors in *Poa Bulbosa*.

Sum of squares *(Poa Bulbosa)*				
Source	df	Root weight	Root dry mass	Root length
Oil pollution	3	5.370**	0.681**	22.278 ns
Stress	3	9.267**	0.534**	11.686**
Oil pollution*Stress	9	2.355**	0.201ns	8.762**
Error		0.863	0.110	1.732

*, ** Show the ($p < 0.05$, $p < 0.01$)

Table 5. Mean comparison of oil pollution application on *Poa Bulbosa* root yield.

Treatment	Root wet weight (Per / Plant)	Root dry mass (Per / plant)	Root length (Per / plant)
Oil (0% w/w)	5.78 c	1.68 b	8.36 b
Oil (1% w/w)	6.11 bc	1.77 b	7.59 c
Oil (2% w/w)	6.33 ab	1.84 b	8.54 b
Oil (3% w/w)	6.75 a	2.02 a	9.62 a

*Values within the same column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Table 6. Mean comparison of Stress application on *Poa Bulbosa* root yield.

Treatment	Root wet weight (Per / Plant)	Root dry mass (Per / plant)	Root length (Per / plant)
FC	5.53 c	1.66 c	8.86 a
FC (75%)	6.81 b	1.97 bc	8.76 b
FC (50%)	6.43 ab	1.86 ab	8.86 a
FC (25%)	6.21 a	1.8 a	7.62 a

*Values within the same column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Significant reduction of plant biomass by the presence of petroleum hydrocarbons has also been reported by Merkl *et al.*, (2004).

Additionally, germination reduction and delay were observed by Adam and Duncan (2002). Inhibition of plant growth parameters (Germination, plant length, and biomass) can be caused by toxic compounds of petroleum hydrocarbons (Bossert and Bartha, 1985). Salanitro *et al.*, (1997) reported seedling emergence reduction of corn, wheat, and oat in soil contaminated with heavy crude oil. A reduction of germination by 30% to 90% for some native species of Mexico in petroleum-polluted soil was also observed by Gallegos-Martinez *et al.*, (2000). Oil components can enter into the seed and disturb metabolic reactions or even kill the embryo (Adam and Duncan, 2002).

Effect of treatments on Poa Bulbosa root wet and dry mass

Result showed that treatments have effect on root biomass, dry mass and length that significant at ($p < 0.01$) table (4). The efficiency of root was in level 3% oil pollution that increasing traits than the no pollution about 16.8%, 20.2% and 15.1% respectively (Table 5, Figure 5).

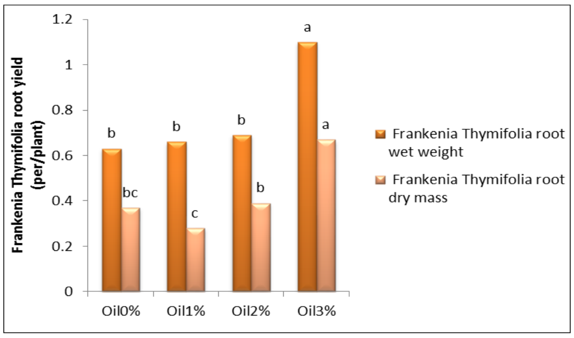

Fig. 1. Effect of oil pollution on *Frankenia Thymifolia* root.

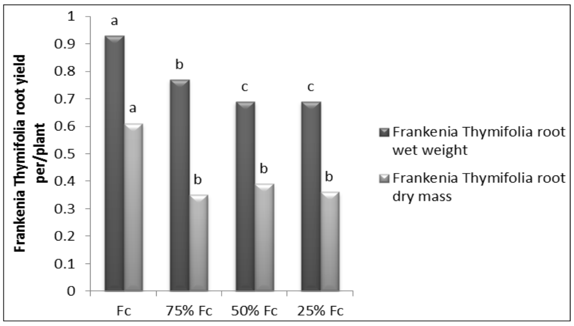

Fig. 2. Effect of stress on *Frankenia Thymifolia* root.

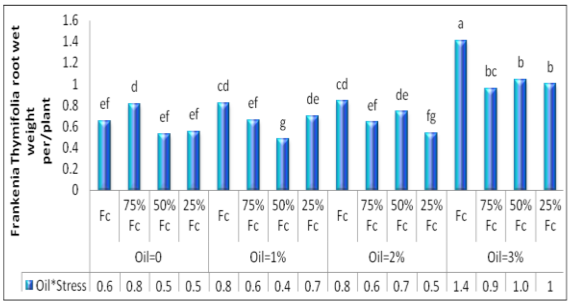

Fig. 3. The interactive of oil pollution and stress on *Frankenia Thymifolia* root weight.

From stress treatments similar results indicate (Table 6, Figure 6). Interactive effect of oil pollution and stress was significant at ($p < 0.01$) (Table 4). Mean comparison results showed that the most effective treatment on root wet and dry mass was 3% oil pollution and $3/4$ stress (75% FC) on root dry that increased it to 44.6% and 44.4% than the no stress and no oil pollution. Effect of other treatments on root wet and dry mass is shown in figures 7 and 8. The effect of treatments on root length was significant at ($p < 0.01$). The results showed the most root length was made with no stress (FC), $1/2$ stress (FC = 50%) and $3/4$ stress (FC= 75%) treatments. And the lowest root length made with $1/4$ stress (FC = 25%) treatment.

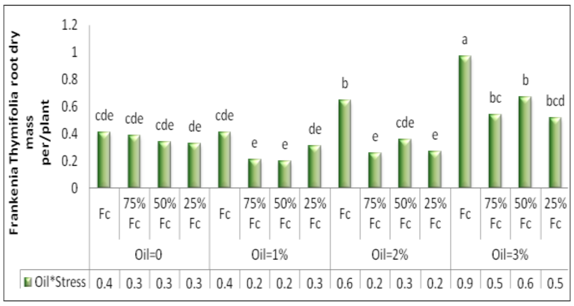

Fig. 4. The interactive of oil pollution and stress on *Frankenia Thymifolia* Root dry mass.

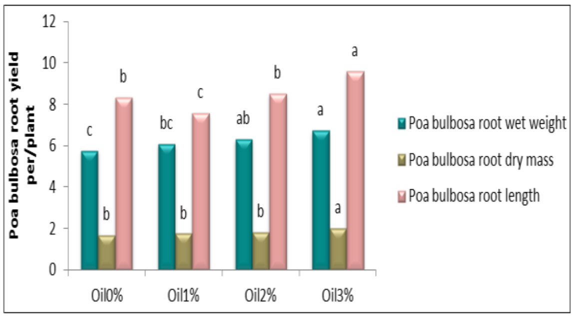

Fig. 5. Effect of oil pollution on *Poa Bulbosa* root.

The interactive effect of crude oil and stress on root length significant at ($p < 0.01$) that shown the (3% Oil, 50% FC) and (3% Oil, 75% FC) 30.2% increased than the no stress and no oil pollution. The lowest length was made by 0% oil and 75% FC. Dominguez-Rosasdo *et al.*, (2004) reported that seed germination of several grass, legume and cereal species declined with an increase in used oil concentration, at oil rates than 1% (w/w). They stated in terms of both germination and overall growth, leguminous plant were generally more resistant to used oil contamination than non-leguminous species mainly due to massive root system.

Merkl and Schultze-Kraft (2005) reported that legumes died within 6 to 8 weeks in heavily crude-oil contaminated soil, whilst the grasses showed reduced biomass production. Furthermore, a positive correlation between root biomass production and oil degradation was found. The microbial communities and their Degradative potential in Rhizosphere of alfalfa, reed and un-planted soil in response to bitumen contamination of soil reduced the total number of microorganisms more significantly (by 75%) in un-planted than in Rhizosphere soil (by 42% and 7%) for reed and alfalfa, respectively (Muratova *et al*., 2003).

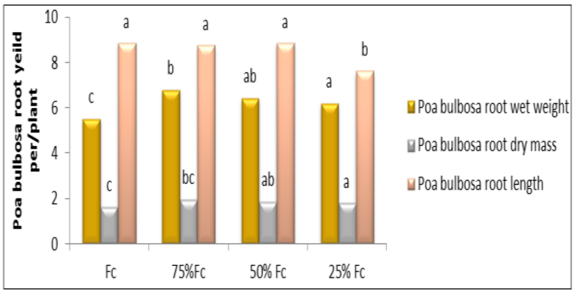

Fig. 6. Effect of stress on *Poa Bulbosa* root.

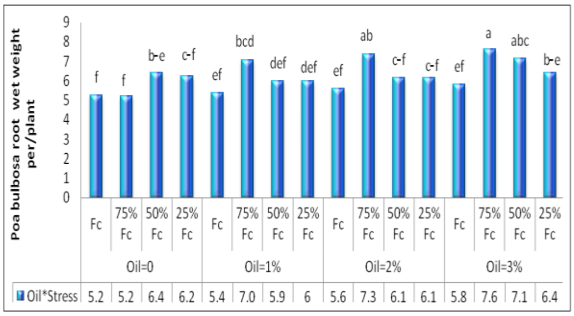

Fig. 7. The interactive of oil pollution and stress on *Poa Bulbosa* Root weight.

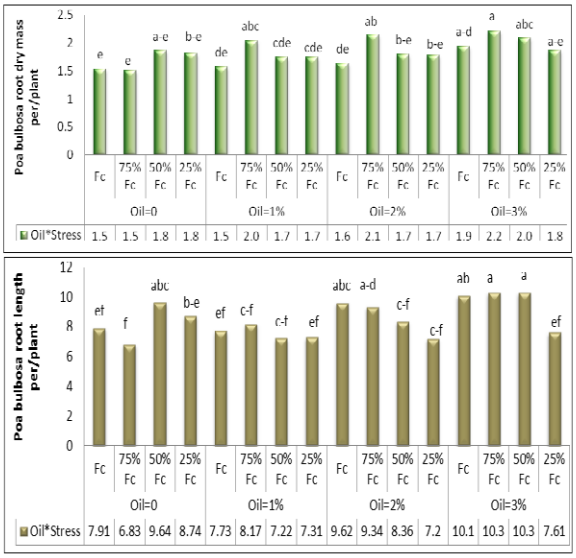

Fig. 8. The interactive of oil pollution and stress on *Poa Bulbosa* Root length.

Merkl *et al.*, (2005), while studying root morphological characteristics of three tropical graminoids (*Grachiaria brizantha*, *Cyperus aggrgatus* and *Eleusine indica*) reported that *G. brizantha* and *C. aggrgatus* showed coarser roots in polluted soil than control as expressed in an increased average root diameter. *G. brizantha* had a significantly greater specific root surface area in contaminated soil. Additionally, oil contamination caused a significantly smaller specific root length and root surface area in the *C. aggrgatus*.

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