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Evaluation of Petroleum Hydrocarbons Uptake by Fetsuca and Geranium: A Case Study at Gasoil Station in Jajrood, Tehran, Iran

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Derived compounds leakage of petroleum by pipelines, overflowing of reservoirs, carrier vehicles accident and petroleum depots that burned in wars are most siginificant sources of environmental pollution by petroleum hydrocarbonsThe fate of spilled petroleum after releasing to environment is very variable, and different factors for controlling the situation are effective. Physical and chemical properties of these compounds, rate of concentration variation related chemical reactions or microbial degradation, environmental conditions such as temperature, light, oxygen and solubility rate in water can be considered. Nowadays, applying methods in accordance with environmental criteria expanding vastly and one of well-studied technique for cleanup of petroleum compounds is utilization of native plants. In current study, uptake of total petroleum hydrocarbons (TPH) by Fetsuca and Geranium with aim of TPH reduction from gas oil polluted soil was evaluated. Results indicated that Fetscua is capable of TPH uptake. Moreover, TPH content in Fetsuca were more than Geranium. With respect to results of this study, both plants can be potential candidate for TPH uptake from soil, where rate of uptake by Fetsuca is higher than Geranium.

Key words: Phytoremediation, Fetsuca, Gasoil, Geranium, TPH.

Continuous development of industries and fossil fuel consumption in current year created irrecoverable pollution in environment. Based on US EPA reports, annually 6 million ton petroleum pollutants release to environment and risk life of fauna and flora. The biggest environmental disaster till now occurred in Gulf Mexico, which seriously affected ecosystem of the region. In this context, phytoremediation of petroleum compound received more attention by many researchers. Some of compounds such as chloride solvents and aliphatic hydrocarbons have moderate to high solubility in water, thus easily transport via soil particles and enter to groundwater. On the contrary, others have no solubility in water and absorb to soil particles and characterized as soil and sediment pollutants. The concept of total petroleum hydrocarbon (TPH) includes a broad range of chemical compounds, which derived from petroleum. This group of chemical compounds are seriously dangerous, thus if released to environment, life of many organisms will be threaten. Refinery operations of petroleum, leakage from pipelines/reservoirs, and accidents related to liquid fuel carriers cause releasing of these compounds to environment and TPH are amongst main pollution sources. Based on accomplished studies TPH is mixture of hydrocarbons which, from point of chemical structure and biological are very persistent and dangerous, respectively. Compounds such as Alkanes, Alkenes, and Aromatic Hydrocarbons have been detected in this group. Alfalfa showed normal growth in soils contaminated with 500 mg/kg Tolouns.

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MATERIALS AND METHODS

The soil samples (from 0-30 cm depth) were collected from a fuel station, in JAJROODof TEHRAN where a lot of gas oil released. (Contaminated soil, referred hereafter as C2). Clean soil (referred hereafter as C0) was derived from a non-polluted area close to the station. Samples were transferred to greenhouse of JAJROOD Municipality, where samples dried and sieved through a 4mm stainless mesh.

Soil preparation

In order to have different level of pollution and reduce side effects of organic pollutants of samples and plants growth,beside to analyze the effect of different concentration of pollutant in samples, soils were treated in order by following procedure. First, soils were passed through 4 mm stainless mesh and stored in greenhouse for 21 days in natural conditions, whereas every day for aeration, mass of soils were displaced. Plastic pots (2kg) were considered for planting to avoid any leaking.

Clean soil (referred hereafter as C0) was derived from a non-polluted area close to the station

Half-polluted soil (w/w 1:1), which prepared by mixture of polluted and clean soil (C1)

Contaminated soil (w/w 2:1), which include 2 part polluted soil and 1 part clean soil (C2)

In order to have homogenous samples and uniform distribution of petroleum pollutants in soils, each of mentioned samples, were stored for 21 days whereas soil moisture maintained in field capacity (FC) and displaced. After 21 days, samples prepared for experiments and transplantation, Thus, Geranium's stem and Festuca seeds transplanted in three different levels of pollution (C0, C1, and C2) and in three replicates at depth of 3 cm of soil surface.

Every morning and afternoon the pots irrigated, whereas after two weeks irrigation was within a day. Festuca growth was extremely marvelous and Geranium at C2 showed reasonable growth. After 60 days, when plant growth completed, weeds in Festuca removed. Total numbers of pots for two plants were 18 which, by a simple randomized block design, final samples for further experiments collected (Fig 1). Totally, 13 samples (6 plant samples and 7 soil samples) transferred to laboratory. In addition, physical and chemical characteristics of soils are shown in Table 1.

Determination of petroleum hydrocarbons in soil:

In order to estimate total concentration of Petroleum Hydrocarbons (TPHs) and some of Poly Aromatic Hydrocarbons (PAHs) in soils, first extraction based on method by X with equal ratio of N-Hexane and Di-Chloromethane (USEPA method No. 8100) was performed. Thus, from each soil sample, 10 g weighted and placed in Thimble paper. Then, samples placed onto flask of Soxhlet extractor. From each solvents (of N-Hexane and Di-Chloromethane) 150 ml added to flask of instrument and temperature adjusted at 70 ºC, where solvents start to evaporate and enter to the condenser. Solvent vapor in the distillation flask started to be cooled and liquid form fallen to soil samples. The procedure continues till depletion of extractors. In fact each depletion count a Soxhlet cycle, which lasts about one to two hours. During each cycle, a portion of organic compounds in soil samples transferred to the flask that due to temperature difference of solvent evaporation and extracted composite, the next cycle of Soxhlet only solvents being passed between evaporation and condenser, whereas extraction remains inside the flask. After completion of extraction, stored dark liquid in flask is concentrated and solvents evaporated until only extract of samples yielded. After condensation of samples and removing of solvents, flask of final extract was weighed and by considering its dried weight, total petroleum hydrocarbon calculated. Results of TPHs measurement in soil samples of different treatments are shown in Table 3. Also total petroleum hydrocarbons in plants in Table 4 presented. Moreover, concentration of some Poly Aromatic Hydrocarnons (PAHs) in contaminated soil has been estimated by GC and based on method 831 provided by USEPA. Outcomes of analysis are shown in Table 2.

Phytoremediation analysis

Plant growing and conservation

In order to evaluate phytoremediation, from each studied soil samples about 2 kg filled in pots (diameter 150 mm, height 250 mm). Next step, Festuca and Geranium transplanted at 1

to 2 depth of pots surface. For each samples of current study, control sample (without plant) to eliminate environmental effects on concentration of petroleum pollutants included. Plant growth lasted 18 weeks whereas Geranium at all treatments had a fall but both of plants growth well and weeds removed. At this step, irrigation of plants similar to previous step fulfilled. At the end of period to estimated TPHs, soil samples at plant treatments collected from root area and bottom of sandy surface for control where concentration of TPHs in each soil of defined treatment analyzed based on Soxhlet method. Amount of pollutants in soil samples in Table 3 – 5 and total petroleum hydrocarbons for both plants in Table 3 – 6 are provided.

Table 1. Some physical and chemical characteristics of studied soils

Physical and chemical characteristics										
Na $^{1-}$mgkg	K $^{1-}$mgkg	P $^{1-}$mgkg	Ca mgkg^{-1}	ECe dS/m	pH	TN %	OM %	$CaCO_3$ %	Clay %	Treatment
31	144.8	150	2158	9.8	6.9	1.22	10.23	24.5	22	C_x
15	121.5	45	2656	7.4	7.8	0.08	0.99	29.5	26	C_o
18	125.0	73.5	2210	7.8	7.7	0.63	4.68	23.7	23	$_1C$
26	128.2	123.5	2848	8.1	7.1	0.87	7.49	20.5	20	$_2C$

* TN = Total Nitrogen / OM = Organic Matter

Table 2. Total petroleum hydrocarbons concentration in soil of different treatments

Treatment	TPHs (mg kg^{-1})
C_0	50>
C_1	150.3
C_2	965.21

Table 3. Total petroleum hydrocarbons concentration in soil

(2:1) Polluted	(1:1) Half-Polluted	Control	TPH/ Soil
106.50	42.80	3.12	Geranium
178.20	26.08	9.46	Festuca

Table 5. Effective factors

		Value Label	N
Replication	1		6
	2		6
	3		6
Soils	1	C0	6
	2	C1	6
	3	C2	6
Plants	1	Geranium	9
	2	Festuca	9

Statistical design and statistical analysis

In germination and primary growth studies split plots experiment in a completely randomized design, for phytoremediation factorial experiment in a completely randomized block design with

Table 6. Dependent variable of TPH in soils

Source of variation	Sum of squares	Degree of freedome	Average of squares	Calculated F	Sigficancy
Soils	61838.360	2	30919.180	209112.759**	0.000
Plants	1923.860	1	1923.860	13011.463**	0.000
Plants/Soils interaction	6255.678	2	3127.839	21154.218**	0.000
Repeatability	0.578	2	0.289	1.954n.s	0.192
Error	1.479	10	0.148		
Total	70019.955	17			

R Squared = 1.000 (Adjusted R Squared = 1.000) ** Significance at 1%

Table 7. Paired comparisons in soils

Control soil Compared soils		Difference of compared average	Standard error	Level of significance(a)	95% confidence interval for comparison between pairs (a)	
					Upper Bound	Lower Bound
C0	C1	-27.825(*)	0.222	0.000	-28.320	-27.330
	C2	-135.892(*)	0.222	0.000	-136.386	-135.397
C1	C0	27.825(*)	0.222	0.000	27.330	28.320
	C2	-108.067(*)	0.222	0.000	-108.561	-107.572
C2	C0	135.892(*)	0.222	0.000	135.397	136.386
	C1	108.067(*)	0.222	0.000	107.572	108.561

Based on estimated marginal means
* The mean difference is significant at the .05 level.
(a) Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments)

Table 8. Paired comparisons in plants

Control Plant	Compared Plants	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
					Upper Bound	Lower Bound
Festuca	Geranium	20.677(*)	0.181	0.000	20.273	21.081

Based on estimated marginal means
* The mean difference is significant at the .05 level.
(a) Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Fig. 1. Festuca and Geranium pots

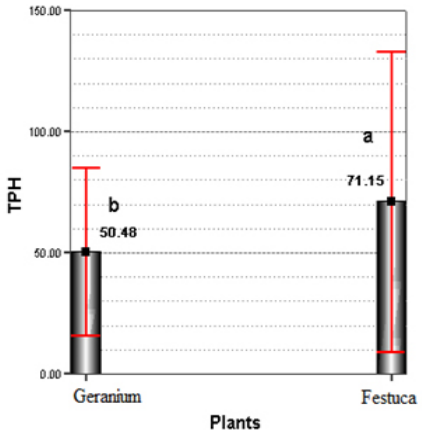

Fig. 2. TPH levels in soil samples

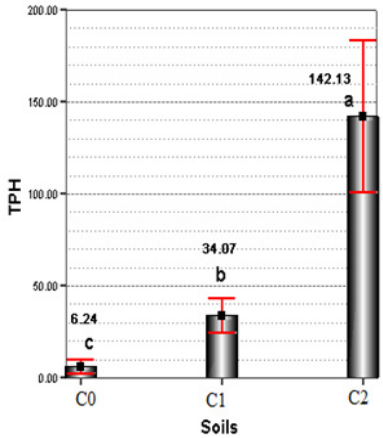

Fig. 3. TPH levels in cultivated soils

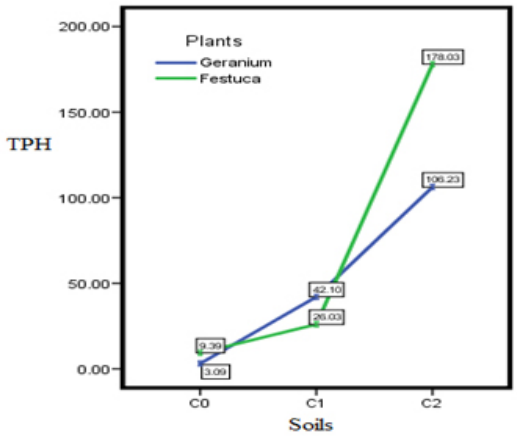

Fig. 4. Effect of soil and plant interactions on TPH changes of soil

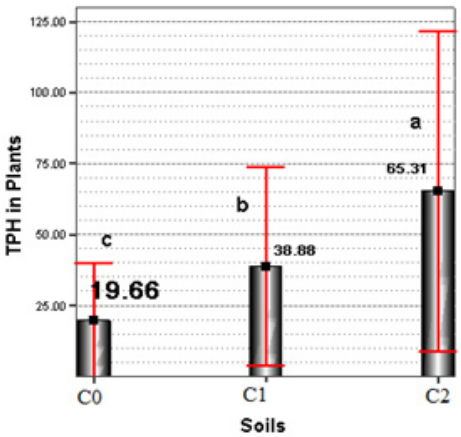

Fig. 5. TPH levels in plants cultivated in different soils

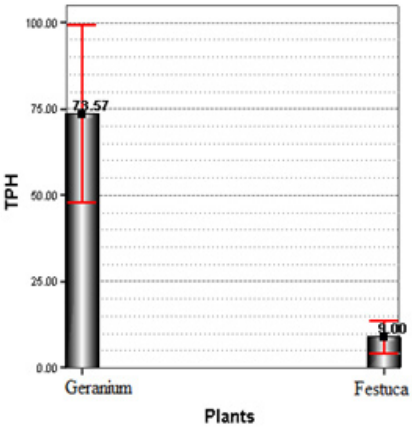

Fig. 6. TPH levels in cultivated plants

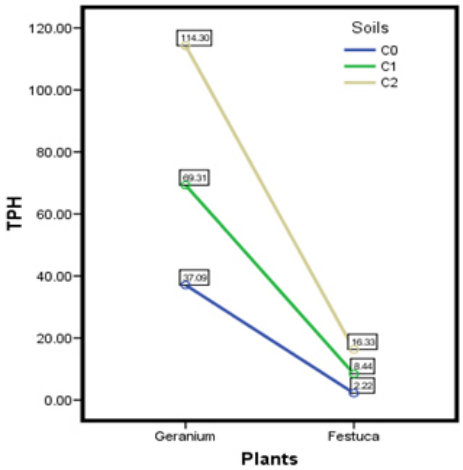

Fig. 7. Effect of soil and plant interactions on TPH changes of plant

2 factors of plant at 3 levels and pollution concentration at 3 levels with 3 replicated, and comparison of averages within LSD method were used. Statistical analysis of data by SPSS (t-test) and for drawing Excel was used. In Table 5 effective factors are shown.

In table 6, variance analysis showed that TPH content of soil significantly at 1% and likely 99% affected by soil and plants factors and may be their interaction. Studying paired comparison of TPH average in soils showed that between soils C0 and C1, C0 and C2, and moreover C1 and C2 significance differences at 5% and probability of 95% existed where soil C2 has the highest TPH, which is shown in Table 7. Paired comparison of plants in Table 8 indicated significant difference in TPH content of soil whereas cultivated soil with Festuca has more TPH (Probability 95%). Results showed that soils of pot C2, which Festuca has been transplanted more TPH observed related to other treatment.

Figure 3 shows that control soil and C2 under cultivation of Festuca had more TPH but C1 under cultivation of Geranium had highest TPH, which is because of soil and plant interaction. Effect of soil and plant interactions on TPH changes of soil are shown in fig4. TPH levels in cultivated plants are shown in fig 5 and TPH levels in plants cultivated in different soils are shown in fig 6 and Effect of soil and plant interactions on TPH changes of plant. Fig 7 are shown Effect of soil and plant interactions on TPH changes of plant.

Figure 3 shows that control soil and C2 under cultivation of Festuca had more TPH but C1 under cultivation of Geranium had highest TPH, which is because of soil and plant interaction.

Figure 6 indicates that Geranium in polluted soils relative to Festuca uptakes more TP.

CONCLUSION

By considering that environmental pollution in soils can easily be transferred to food webs, it becomes necessary to apply phytoremediation and green methods to protect the environment and prevent pollution. The current study shows Geranium and Festuca, as fast growth plants and having a high uptake of TPH can be potentially used in contaminated sites. As it can be observed in Figure 5, TPH levels for Geranium is higher than Festuca at C2. Therefore, transplantation of discussed plants around fuel stations and site with possible leakage of oil, gasoil, and derived compounds is highly recommended.

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