Original Article

Feasibility of Sunflower (*Helianthus annus* L.) Plantation in Low to Moderately Contaminated Brownfields to Achieve Remediation Objectives

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Abstract

Phytoremediation has gained increasing attention in recent years because of its ability to clean up and redevelop brownfield sites. Petroleum hydrocarbon-contaminated sites are among most prevalent industrial sites in Iran as one of the top oil producers in the world, which are in great need of remediation to prevent associated environmental and human health risks. A greenhouse study was conducted to identify the tolerance of sunflower (*Helianthus annus* L.) and its growth parameters under various scenarios. Additionally, remediation potential of sunflower in spiked soil with low to moderately gasoline-contaminated soil was evaluated. Results indicated that sunflower is a tolerant plant species in low to moderately gasoline-contaminated soil with remarkable biomass establishment in contaminated soil and acceptable phytoremediation potential. Sunflower was found to tolerate gasoline contamination up to the maximum applied concentration of 5000 mg/kg. However, sunflower biomass and height slightly decreased in presence of gasoline in soil which were not significant in most cases ($P>0.05$). Significant reduction of gasoline under the influence of sunflower was obtained in all vegetated treatments when compared to non-vegetated treatments ($P<0.05$). Phytoremediation effectiveness of sunflower did not vary significantly with increasing contamination level in soil ($P>0.05$). Results of the present research indicated that gasoline content of soil cannot be considered as a controlling factor affecting phytoremediation potential of sunflower when soil is polluted with low to moderate levels of gasoline. Soil remediation with sunflower can be considered as a promising approach to manage moderately gasoline-contaminated sites.

Keywords: Gasoline, Phytoremediation, Plant Growth, Spiked Soil, Sunflower

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Introduction

Gasoline is a complex mixture of hundreds of hydrocarbons with different properties that does not naturally exist in the environment [1]. The actual composition of gasoline may vary with the source of crude petroleum, refining process, and the time of year. Adverse human health effects of exposure to gasoline have been well documented in the literature [2, 3]. Some chemicals in commercial gasoline may evaporate, some might dissolve in and be transported by water, and the rest probably stick to soil particles. People can be exposed to gasoline by different pathways such as inhaling evaporated components at a gas station or affected site and drinking contaminated water [1, 4]. Furthermore, gasoline components in soil will not remain at the affected point and can be mobilized by water, then pass through soil layers and find their way towards groundwater resources which is of great concern in most gasoline-contaminated sites.

Clean up of polluted sites is a challenging issue throughout the world, which has gained increasing attention in recent decades. There are many suspected contaminated sites in Iran such as oil refineries. However, identification and remediation of such brownfields have not been addressed seriously mainly due to economical and technical restrictions. Brownfields can be defined as a property for which the redevelopment or reuse may be difficult due to the availability or potential availability of hazardous substances or pollutants [5, 6] such as petroleum hydrocarbons. Decontamination of contaminated sites and their redevelopment can bring about a wide variety of benefits to a given community such as new employment opportunities, using available infrastructures, preventing development of greenfields, protecting human health, bring a dead area back to life, and hence improving the life quality [7, 8]. In other words, remediation and redevelopment of contaminated sites located in urban areas are of utmost importance for sustainable urban development. Mean while, several serious challenges including environmental liability concerns, financial barriers, clean up considerations and regulation consistency, and reuse planning for the future of the site make cleanup of contaminated sites and their redevelopment unique compared to other real estate development projects [6].

Biological remediation approached for contaminated soils have been preferred compared to physicochemical methods because they are cost-effective and do not disturb the environment. Among biological treatment methods, plant-aid remediation has gained remarkable attention in recent years in both developed and developing countries. Plant-assisted bioremediation which is mainly known as phytoremediation is defined as the use of plants and their associated microorganisms [9] and can be employed to

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remove a broad range of contaminants from soil such as petroleum hydrocarbons. In general, soil treatment is based on synergistic cooperation between plant roots and soil microbial community. In other words, there are mutual benefits for both plants and microorganisms in the presence of pollutants [10, 11]. Root exudates and enzymes can supply nutrients to microorganisms and plant species can benefit from the capability of microorganisms to detoxify pollutants which in turn reduce the stress on plant growth in contaminated soil [12]. Dissipation of organic contaminants such as gasoline in soil during a phytoremediation process is mainly associated with the stimulation of microbial activity by root exudates i.e. rhizodegradation or phytostimulation [13].

Successful remediation of various organic contaminants like petroleum hydrocarbons has been reported in the literature [11, 14-18]. Phytoremediation potential of *Lolium perenne* has been evaluated in a soil contaminated with 2.8% petroleum hydrocarbons in a greenhouse experiment by Masu *et al*., (2013) showed promising results [19]. In another study, the influence of alfalfa on degradation of total petroleum hydrocarbons (TPHs) was evaluated for artificially contaminated soils by Gouda *et al*., (2016). Their findings showed significant removal of TPHs from soil in presence of alfalfa, compared to non-vegetated treatments [20]. In spite of a numerous successful experiments, contradictory results have also been reported in which removal of organic contaminants such as hydrocarbons from soil was not significantly affected in presence of plant species in soil [13]. Plant growth suppression in presence of hydrocarbons in soil was the most significant cause affecting phytoremediation capability of a given plant species. Furthermore, it should be noticed that hydrocarbon degradation and removal from soil may vary from one plant species to another one as phytoremediation is a site-specific remediation approach [11].

Iran encounters various environmental challenges among which soil pollution is of great concern mainly due to the fact that soil pollutants can be mobilized and transported to soil sub layers and affect groundwater resources and also enter the food chain, hence, endanger human health seriously. On the other hand, soil pollution with hydrocarbons has extensively observed in many parts of Iran e.g. near oil refineries and spill sites [21], because of remarkably high rate of oil-related activities in Iran as one of the top oil producers in the world. Widespread demand to remediate soils affected by petroleum hydrocarbons such as gasoline inspires interest on application of environmental friendly and cost-effective remediation technologies in the country. Phytoremediation seems to present a promising solution that has the capability to rehabilitate hydrocarbon affected sites; however, remediation potential of a given plant species in presence of different levels of contaminants in soil is required to be precisely evaluated. The main objectives of the present research are to evaluate growth behavior of sunflower (*Helianthus annus* L.) in low to moderately gasoline-contaminated soils and also to examine the phytoremediation potential of sunflower under various scenarios. Since hydrocarbon content of soil is a crucial factor affecting plant establishment and performance in contaminated soil, phytoremediation experiments were conducted in presence of different levels of gasoline in soil.

Materials and Methods

Clean soil was provided from lands around the Oil Refinery of Tehran, located at south of Tehran, Iran. Air dried soil passed through a 4 mm sieve in order to prevent loss of coarse-grained soil particles and then mixed thoroughly by hand. Selected properties of the used clean soil are presented in Table 1. Clean soil was artificially spiked by commercial gasoline to reach 500, 1000, 2500, and 5000 mg/kg (w/w) contamination levels in soil. Samples were transferred to polyvinyl chloride (PVC) pots and filled to achieve 1000 g of the contaminated soil in every pot. The same amount of soil was used in uncontaminated treatments. All treatments including vegetated and non-vegetated, contaminated and non-contaminated treatments were considered in triplicate.

Since sunflower seeds are relatively large, eight intact seeds of sunflower were planted in every vegetated pot. Appropriate distance between seeds was considered in order to avoid interference effects of adjacent seeds during the growth period. If seeds are planted very closely the above-ground organs of plant may not receive sunlight uniformly and some plants may suffer from the lack of enough photosynthesis that will in turn affect phytoremediation performance of plant species. Additionally, densely planted pots may encounter lack of nutrients during the growth period as the amount of soil and its associated nutrients are confined in greenhouse experiment. In the present study, Sunflower (*Helianthus annus* L.) was cultivated for over a two months period in greenhouse under natural sunlight. Germination of sunflower was monitored daily to reach the final germination rate. In most cases, final germination was achieved during the first week. Of course, onsite observations however, onsite observations showed delayed germination of sunflower in some cases. Seedling emergence in all vegetated treatments did not change after 10^{th} day from the start of the experiment. Number of emerged seeds was counted and presented as percentage by dividing to the initial number of implanted seeds.

Table 1. Physicochemical properties of the uncontaminated soil.

Parameter	Value	Analytical method
Clay (%)	36	Hydrometer measurement
Organic matter (%)	0.9	Walkley-Black
Soil pH	7.2	1:1 soil/water slurry
Electrical Conductivity (dS/m)	3.1	1:2 soil/water slurry
Total N (%)	0.12	Kjeldahl
Phosphorus (mg/kg)	29.3	Olsen

Plant biomass and root height was measured in 20 day intervals. Destructive pots were considered in order to measure root and shoot of harvested plants in mentioned intervals. Underground organs were carefully removed from soil and washed with deionized water to remove soil

particles from plant tissues and air dried for half an hour. Air dried plant organs were oven-dried in 70°C for 48 hours in order to measure dry biomass of sunflower shoot and root in different treatments.
Soil samples were taken every 20 days from pots and air dried at room temperature, then passed through a 2 mm screen. Samples were refrigerated at 4ºC before being extracted and analysed for residual concentration of gasoline in samples. Hydrocarbons were extracted using dichloromethane solvent. Concentrated extract was injected into a gas chromatograph equipped with a flame ionization detector (FID) in order to determine residual gasoline concentration in soil samples. Results were analyzed using IBM SPSS Statistics 24. Significance level was considered at $p = 0.05$. Significance of differences was examined using one-way analysis of variance (ANOVA), and then, if needed, least significant difference (LSD) test was conducted.

Results and Discussion

Sunflower was chosen as the subject plant species in this research as it has been observed to grow wildly in contaminated lands around the Oil Refinery of Tehran. The ability to tolerate harsh conditions under environmental stress is an important required characteristic for a plant to be selected for remediation purposes [10]. In addition sunflower is a fast growing species that makes it suitable for phytoremediation intentions as establishment of considerable biomass is a crucial factor affecting phytoremediation potential of a given plant species [9]. Results of the present study demonstrated that contamination of soil with hydrocarbons could not inhibit germination of sunflower in presence of different applied concentrations of gasoline. Sunflower showed to be a tolerant plant species to hydrocarbon contamination of soil in this study. Contamination of soil with gasoline did not have significant influence on germination rate of sunflower in presence of 500, 1000, and 2500 mg/kg gasoline ($P>0.05$); however, seedling emergence reduction was significant compared to the control clean soil ($P<0.05$) when 5000 mg/kg gasoline was added to soil. Final seedling emergence of sunflower which was monitored on daily basis is shown in Figure 1.

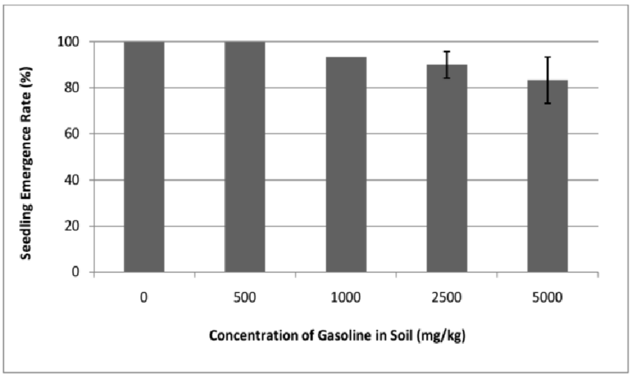

Figure 1. Final germination of sunflower in gasoline contaminated soils. Error bars represent standard deviation (n=3).

Onsite observations showed that germination rate remained constant after ten days. Sunflower germination rate was reduced by only 16.7% in soil contaminated with 5000 mg/kg. Susceptibility of seedling emergence and subsequent initial growth of plant species can influence the phytoremediation performance of a given species [22]. In other words, weak germination might be followed by poor establishment of plant in contaminated soil [23]. Germination rate of sunflower did not fall below 83% in any of the contaminated treatments in the present study that indicates tolerance of sunflower seeds to germinate in low to moderately gasoline-contaminated soils.
Delayed emergence of blossom was observed when 5000 mg/kg gasoline was added to soil. In general, germination of a given plant species in contaminated sites is more susceptible to freshly applied hydrocarbons compared to aged pollution and toxic effects of freshly added hydrocarbons on plant germination and growth might be more severe than aged soils mainly due to the sudden stress caused by concentration variation exerted to plant seed in freshly contaminated soils. Inhibitory effects on plant growth and delay or reduction in germination rate of a wide variety of plant species such as corn, wheat, oat, and vetiver grass in presence of hydrocarbons in soil have been reported in the literature [10, 24, 25].
Root biomass and length and shoot biomass are presented in Table 2. Plant biomass establishment is one crucial factor affecting phytoremediation potential of a given plant species. Above-ground heights of sunflower in different treatments were also monitored. Growth parameters of sunflower did not show considerable change when 500, 1000, and 2500 mg/kg gasoline were added to soil; however, applying 5000 mg/kg gasoline to soil reduced some growth parameters significantly compared to control treatments associated with each applied concentration ($P<0.05$). For instance, root biomass and shoot biomass of sunflower decreased by only 2.4% and 2.9%, respectively, when 500 mg/kg of gasoline was added to soil while addition of 5000 mg/kg gasoline to soil reduced root and shoot biomass by 17.1% and 21.4%, respectively (Table 2). In spite of the fact that obtained reduction in presence of 5000 mg/kg gasoline in soil is statistically significant, it is not practically remarkable such that it can inhibit sunflower phytoremediation performance. Results shows that variation of shoot height of sunflower is less than 10% in presence of 500 and 1000 mg/kg gasoline in soil at the end of the experiment and application of 2500 and 5000 mg/kg gasoline could only reduce above-ground height from 68.7 cm in control soil to 60.7 cm (11.6% reduction) and 58.7 cm (14.6% reduction), respectively.
Reduction in all measured growth parameters i.e. root and shoot biomass as well as plant organs' height was almost negligible in spiked soil with 500 mg/kg gasoline (less than 5% variation at the end of the experiment). Sunflower biomass diminished with increasing content of gasoline in soil almost linearly. For example, addition of 500, 1000, 2500, and 5000 mg/kg gasoline to soil reduced production of dried biomass of above-ground organs at the end of the experiment by, respectively, 3%, 13.4%, 16.4%, and 21.4% in comparison with the control treatment.

On-site observations showed that sunflower does not have extensive and dense root system for both contaminated and uncontaminated treatments. The interesting point which has rarely been addressed in the literature is that comparison between above-ground biomass and under-ground biomass reveals that shoot biomass dominates root biomass in both contaminated and non-contaminated treatments that does not follow the common growth pattern for some other plant species such as grasses which commonly produce higher biomass of root compared to shoot biomass [26], that suggests sunflower is more likely to be effective in removal of inorganic contaminants such as heavy metals compared to organic compounds from soil since removal of heavy metals from soil is more associated with their extraction from soil followed by their translocation to above-ground organs rather than their degradation in the rhizosphere. In other words, great shoot biomass and height of sunflower makes it a probable suitable option for extraction, translocation, and accumulation of inorganic contaminants too.

Table 2. Growth parameters of sunflower for destructive pots.

Parameter	Time (Day)	Initial concentration of gasoline in soil (mg/kg)				
		0	500	1000	2500	5000
Root biomass (cm)	20	2.3	2.0	2.0	1.8	1.6
	40	5.6	5.1	4.9	4.4	4.3
	60	8.2	8.0	7.0	7.1	6.8
Shoot biomass (gr)	20	4.6	4.4	4.0	3.7	3.6
	40	11.7	11.1	10.8	10.4	9.2
	60	20.1	19.5	17.4	16.8	15.8
Root Lenght (cm)	20	8	8	7.5	7	6.5
	40	18	17.5	17	16	15
	60	26	25.5	24	22	20

The highest values of measured growth parameters were obtained for vegetated plants in clean soil (control treatment) in which there was no contamination background. Results indicated that susceptibility of measured parameters i.e. plant biomass and height is almost comparable to each other. For instance, addition of 2500 mg/kg gasoline to soil depressed root biomass, shoot biomass, root length, and shoot height by respectively, 13.4%, 16.4%, 15.4%, and 11.6% compared to growth parameters in clean soil at the end of the phytoremediation experiment. Plant growth started to slow down after 40 days in both polluted and non-polluted treatments suggesting nutrient depletion in confined soil of pots. Nutrient addition to soil through fertilization might enhance sunflower growth in later stages of the phytoremediation experiment. However application of fertilizers does not necessarily enhance phytoremediation performance as asserted by Venosa and Zhu (2003) [27]. In addition, using excessive amount of nitrogen-based fertilizers may result in an enhancement in soil salinity and increase osmotic stress and therefore reduce the activity of hydrocarbon-degrading bacteria in the rhizosphere [28].

Depression of plant growth in the presence of various petroleum hydrocarbons in soil has been reported in the literature. In a study by Brandt *et al*., (2006) considerable decrease in biomass production of vetiver was found in the presence of 5% (w/w) crude oil [10]. Wiltse *et al*., (1998) reported a significant reduction in biomass of alfalfa which is a forage legume when 2% (w/w) petroleum hydrocarbons were added to soil that shows sensitivity of alfalfa to high content of hydrocarbons in soil [29]. In another study Gallego-Martinez *et al*., (2000) also found a depression of plant biomass for three plant species [30]. High rates of plant mortality and decline in plant weight and height are common reactions caused by hydrocarbon contamination in soil. There are reports on large variation of hydrocarbon tolerance among plant species growing in polluted soils [12]. Plant growth depression in contaminated soils might be caused by toxic effects of hydrocarbons in soil. In addition, gasoline usually has more severe effects on plant growth compared to other petroleum hydrocarbon products e.g. heavy crude oil at the comparable levels. It is due to the fact that gasoline generally contains higher contents of volatile and low-molecular weight components which are more toxic to plant health [31]. Despite this fact, sunflower demonstrated to be tolerant to presence of various applied concentrations of fresh gasoline in soil in the present research. Phytoremediation effectiveness of sunflower in various vegetated treatments in addition to natural attenuation of gasoline in non-vegetated soils are presented in Figure 2. It can be inferred from Figure 2 that hydrocarbon concentrations declined significantly in all vegetated and non-vegetated treatments ($P<0.05$) over the duration of the experiment. Considerable influence of the employed plant species on dissipation of hydrocarbons from soil was obtained during the pilot study, especially at later stages of the phytoremediation experiment. This may be attributed to the fact that volatilization of lighter fractions of gasoline may have contributed to the rapid reduction of gasoline content in soil at early stages of the experiment as also suggested in the literature [12]. Microbial degradation can also be associated with reduction in gasoline content of soil in non-vegetated treatments. Both of these actions constitute a major fraction of natural attenuation in soil [12, 32]. Natural attenuation reduced gasoline concentrations in soils spiked with 500, 1000, 2500, and 5000 by, respectively, 30.01%, 28.39%, and 27.43%, and 28.46%, respectively, at the end of the experiment. Hydrocarbon removal rate through natural attenuation process did not vary among different treatments ($P>0.05$). That is to say, natural attenuation efficiency remained rather constant with increasing concentrations of gasoline in soil.

Results indicated that gasoline dissipation in vegetated soils is significantly higher than that of in non-vegetated soils in which hydrocarbons were attenuated naturally in absence of sunflower (Fig. 2).

For instance, hydrocarbons were mitigated by 49.42% in presence of 5000 mg/kg gasoline in planted soil, whereas only 28.46% of hydrocarbons were naturally attenuated in unplanted control soil at the corresponding gasoline concentration that means 1.7 times increase in hydrocarbon dissipation from soil in presence of sunflower. Enhanced degradation of various organic contaminants such as pesticides, and polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, and crude oil in presence of plant species in soil has been reported in the literature [9, 33-35].

The maximum removal rate of hydrocarbons was obtained at the end of the experiment for all treatments. For instance, the highest removal rate of hydrocarbons in vegetated and non-vegetated soils spiked with 2500 mg/kg gasoline reached to 50.75% and 27.43%, respectively, which was obtained at 60th day. Remediation potential of sunflower slightly decreased with increasing initial concentrations of gasoline in soil. However, variation of sunflower performance among different treatments with various levels of gasoline content was not significant (P>0.05). In other words, gasoline removal from soil in presence of sunflower declined from 54.06% (at concentration of 500 mg/kg) to 49.42% (at concentration of 500 mg/kg), that means maximum of 8.6% reduction in phytoremediation effectiveness of sunflower due to increase in initial gasoline content of soil from 500 to 5000 mg/kg. Results of the current research indicate that gasoline content of soil cannot be considered as a controlling factor affecting phytoremediation potential of sunflower when soil is contaminated with low to moderate levels of gasoline.

Slight reduction in phytoremediation effectiveness at higher concentrations of gasoline in soil may be attributed to both the toxic effects of hydrocarbons at higher gasoline concentrations and their influence on plant growth parameters which declined in this study with increasing content of gasoline in soil. Higher initial gasoline concentrations may also affect microbial community in the rhizosphere of sunflower.

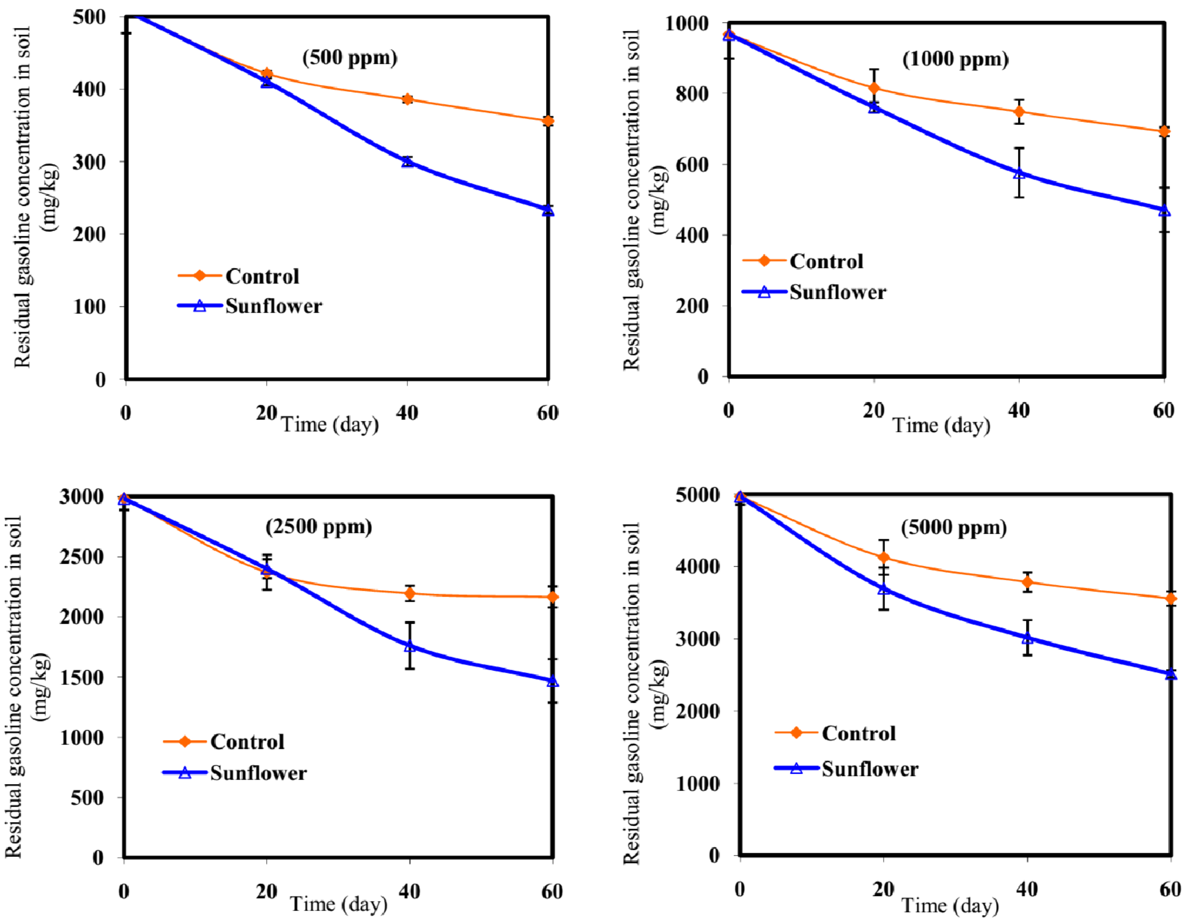

Figure 3. Residual amounts of hydrocarbons in soil during the experiment. Error bars represent standard deviation (n=3).

Cyperus rotundus (Linn.), *Cyperus brevifolius* (Rottb.) Hassk, *Cyperus odoratus* L., and *Cyperus laevigatus* L. showed remarkable removal of hydrocarbons in soil when they planted in 8% petroleum hydrocarbon-contaminated soil in a phytoremediation study. They suggested that the capability of the plant to tolerate the toxicity of hydrocarbons is the main reason for successful phytoremediation [12]. Results of the present study also showed considerable tolerance of sunflower to presence of gasoline in soil, but that could not guarantee the high removal rate of hydrocarbons from soil. Because phytoremediation potential of a given plant species also depends on other factors such as root system and extension. Root proliferation of a given plant species can support a flourishing microbial consortium, that promotes biodegradation of hydrocarbons in soil [36]. Therefore it can be assumed that higher production of root biomass means a larger rhizosphere for microbial population and it can be correlated with a higher degradation of hydrocarbons in soil [11, 31].

Gasoline removal rate in vegetated treatment diminished at later stages of the experiment i.e. after 40th day which may be attributed to the reduction in plant growth rate. Non-appreciable hydrocarbon removal was also observed in a phytoremediation study by Escalante-Espinosa *et al*., (2005) from 120 to 180 days of growth in both vegetated and non-vegetated treatments [17]. Another explanation for reduction of hydrocarbon dissipation rate might be a probable variation in amount and composition of root exudates at later stages of sunflower growth [37].

It is suggested in the literature that degrading bacteria in the rhizosphere can be stimulated by root exudates that vary with plant age and nutritional status [10, 11]. Change in composition of plant exudates with plant age can affect activity of hydrocarbon degrading microorganisms in the rhizosphere, thereby, affect remediation performance of a given plant species. Based on the obtained results, sunflower can be introduced as a tolerant phytoremediator plant species; however, its real-world performance should also be investigated. In addition, autoclaved soil can also be examined to get insight into real effetiveness of sunflower in gasoline removal from soil.

Conclusion

Based on the obtained results sunflower can be introduced as a tolerant plant species in soils contaminated with low to moderately contaminated soils. Addition of up to 5000 mg/kg gasoline to soil did not affect sunflower growth parameters considerably in most cases. Enhanced removal of gasoline from soil in all vegetated treatments compared to non-vegetated treatments was obtained that might be attributed to promising tolerance and growth of sunflower, which is a native and accessible plant species in Iran, in contaminated soil.

Gasoline dissipation from naturally attenuated treatments as well as vegetated treatments did not change significantly with variation of gasoline concentration in soil. However, phytoremediation potential of sunflower slightly declined by increasing content of spiked gasoline in soil and the highest performance of sunflower achieved in presence of 500 mg/kg gasoline.

Sunflower established remarkable biomass and showed acceptable phytoremediation potential in this study. However, remarkable shoot biomass production as well as onsite observations of root extension pattern in destructive pots strengthening the hypothesis that sunflower may also be effective in remediation of contaminated soil with inorganic contaminants such as heavy metals since the main mechanism involved in remediation of heavy metals is based on extraction of contaminants from soil (phytoextraction) followed by translocation and accumulation of them in above-ground organs. Based on the obtained results, sunflower could establish considerable shoot biomass; but sunflower root system was not as extensive/dense as some other phytoremediator plant species such as tall fescue grass. That is to say, results of this research demonstrated that sunflower is a tolerant plant species in gasoline-contaminated soils but plant ability to germinate and survive in contaminated soils is not the only required criterion to be considered in selection of phytoremediator plants and, hence other factors such as root system as well as composition of root exudates and their interaction with target contaminants should also be taken into consideration for real-world phytoremediation purposes. In addition, aapplication of higher gasoline concentrations in future studies can gain insight into the tolerance of sunflower to higher contamination levels.

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