Physiological responses of *Polypogon monspeliensis* L. in petroleum-contaminated soils

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Abstract

In a greenhouse study, decontamination capacity of the species *Polypogon monspoliensis was* investigated for detoxification of petroleum-polluted soils caused by sewage and waste materials of Tehran Petroleum Refinery. For this purpose, the amount of total oil and grease before and 45 days after transplanting one-month-old seedlings in the soils of five different treatments were measured. Pollution-free agricultural soil and contaminated soil were mixed together with the weight ratios of 1 to 9 (%10), 2 to 8 (%20), 3 to 7 (%30), 4 to 6 (%40), and 5 to 5 (%50). These were then compared with the amounts obtained from control treatment without vegetation, but with the same concentration of pollution. Findings demonstrated that the maximum reduction in the petroleum rate (84.85%) was related to the treatment of 10% contamination containing the plant. Increase in the shoot height in treatments 10% and 20% as well as the root dry and fresh weight in treatments 10% , 20% , and 30% shows that probably activity of more rhizosphere microorganisms of the plant in these treatments has led to the improvement in the growth of plant organs comparing to the treatments without pollution.

Keywords: *phytoremediation; total oil and grease; rhizosphere; microorganisms; petroleum-contaminated soil*

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Introduction

Nowadays environmental pollution is one of important issues with which different communities are facing. Contaminants are one of the factors that create disorders in the environment and water, soil, and air as important components of environment are directly exposed to the pollution resulting from industrial activities. The existence of soil and water contaminated with petroleum is one of the common problems in the areas around oil sites and refineries (Golestani and Farajolah Zadeh, 2010). Pollution of soil with petroleum hydrocarbons is a very important environmental issue that remarkably has been taken into consideration in recent decades (Kamath et al., 2004). Pollution of soil with petroleum through oil chemicals and its extraction is of global importance (Li et al., 2002). Petroleum hydrocarbons are a mixture of chemicals which

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are produced using oil products in industry and agriculture, some of which are composed of two benzene rings or more. The collection of hydrocarbons is one of the major groups of contaminants present in the environment and are toxic to the survival of most organisms (Huang et al., 2005). In addition, these pollutants can be absorbed into the surface of soil particles or organic particles existing in the soil and gradually increase in their concentrations and along with surface currents come into surface waters. On the other hand, these compounds may find their way into underground waters along with currents of deep waters and finally they will be able to enter the food chains of plants, animals, and humans and toxify the living beings (Rababah and Matsuzawa, 2002). Therefore, oil hydrocarbons in the soil are not only considered as a harmful factor for human health , but also a negative stimulus for growth and development of plants and animals. (Joner, 2004; Das and Mukherjee, 2007). In general, concentration of a lot of petroleum hydrocarbons (PHCs) in the environment threatens human health and ecosystem security including prevention of plants from growing, destruction of soil structure, and damaging the quality of underground waters (Meagher, 2004).

Soil pollution with petroleum hydrocarbons takes place as the result of extraction, accidents, transportation, leakage from tanks, cuts or breaks in pipelines refining, and consumption of oil products (Scott, 2004) Iran is one of the oil-rich countries in the world extracting a great deal of petroleum every year from southern regions and refining it in other areas. Most of the petroleum and refineries in Iran are located in the urban areas or the regions with agricultural activities. Tehran petroleum refinery, located in south of Tehran, is surrounded by agricultural lands and it is one of the oldest refineries in Iran. Because of oil pollution in some parts of this area, the ecosystems here are faced with serious challenges. As a result, the significance of detoxification of soils which are in contact with oil products and also their transfer is not only an important environmental issue, but also it is significant regarding the protection of agricultural products (Besalatpour et al., 2008).

Today many common methods are used for refining and decontamination of water and soil polluted with toxic compounds. However, America Environment Protection agency has introduced detoxification at the very same polluted site as the most appropriate and the most economical approach (Besalatpour et al., 2008).

Using biological methods including phytoremediation, i.e. using plants to clean up polluted lands, as options which are acceptable economically, has been taken into consideration and has been raised as a new approach in cleaning up polluted soils and waters (April and Sims, 1990). Compared to biological methods, physical and chemical ways are too expensive and may cause secondary pollutions in the soil or water, which as a result, still require further decontamination. Phytoremediation is a modern and advanced technology in which plants are used to remove pollution away from soils, underground waters, sediments, and surface waters. Phytoremediation involves different techniques taking the advantages of vegetation, relevant enzymes, and other complex processes (Russell, 2005). This new method has a great potential for removing pollution from sites and also is a low-cost technique in which certain plants and bacterial colonies related to their roots in the soil are used for elimination, transformation, or absorption of toxic chemicals existing in soils, sediments, underground waters, surface waters, and even the atmosphere (Susarla, 1970). In this method plant roots using some mechanisms create an appropriate environment for decreasing organic compounds. The plant root system allows a quick movement of water and gases in order to improve the soil structure. In addition, it creates an active biological area in the soil rhizosphere which stimulates microbial activities and increases biological access to pollutants (Newman and Reynolds, 1970)

Materials and Methods

Collection of samples

Samples of pollution-free soil were collected from an agricultural field in the 'Haj Abdal' village located in Basmanj town in Eastern Azarbaijan with geographical features of 37 degrees and 58 minutes north and 46 degrees and 29 minutes east. From the depth of 0 to 20 centimetres of the bed of a lake where sewage and waste materials

Table 1.
Some physicochemical features of soils under study

Evaluated features	Treatment	
	Pollution-free soil	Fully-polluted soil
Percent of clay(%)	20	21
Percent of sand(%)	38	36
Percent of silice(%)	42	43
Soil texture	Loam-clay-loam	Loam-clay-loam
Electrical conduction (dS/m)	2.6	3.6
pH	7.4	7.1
Total nitrogen (%)	0.10	0.24
Percent of organic compounds (OC%)	1.9	9.29
phosphor (ppm)	68.2	139
potassium(ppm)	436	179
sodium(ppm)	92	48
calcium (meq/l)	10.10	2.19
Magnesium (meq/l)	7	6.4
iron(ppm)	9.18	6.83
zinc (ppm)	2.86	15.3
copper (ppm)	1.28	8.6
manganese (ppm)	6.68	4.6

of Tehran Petroleum Refinery were poured, samples of the soil polluted with petroleum hydrocarbons were collected, with geographical features of 35 degrees and 30 minutes north and 51 degrees and 26 minutes east. Both contaminated and pollution-free samples were sifted through 2 millimeter meshes of a sieve. Samples were transferred into a refrigerator and kept at a temperature of 4 degrees centigrade until the beginning of the experiment. The seeds of '*Polypogon monspeliensis'* were provided by the Gene Bank of Forests and Natural Resources Organization located in Peikanshahr, in Tehran Province. The seeds were put into polyethylene bags and were kept in a refrigerator at temperature of 4 degrees centigrade until the beginning of the experiment.

Evaluation of physicochemical features of the soil

To find out the features of both pollution-free soil and the soil polluted with oil hydrocarbons, first the soils were sifted through a sieve with 2 mm meshes. Then, we specified the parameters of the soil texture by hydrometric method (Bouyoucos, 1962) , electrical conduction in the extract of the saturated mud (Richards, 1954), absorbable phosphor by Olsen method (Olsen et a., 1954), absorbable sodium and potassium by the method of extraction with ammonium acetate and reading by flame photometer (Richards, 1954), total nitrogen by Kjeldahl_method, soil pH in the saturated mud by means of pH meter (Mc Keague, 1978 and Mc lean, 1982), percentage of organic materials by oxidation method (Walkley and Black, 1947; Fao, 1974), amount of soluble calcium and magnesium by titration method (Richards, 1954), and amount of micronutrients of iron, zinc, copper, and manganese by atomic absorption method (Lindsay and Norvell, 1978). The experiments related to the determination of physicochemical features of soil were carried out by 'Pars Dariun Development Engineering Services Company'. The results obtained from measuring the physicochemical features of both oil-polluted and pollution-free soils are shown in Table 1. The results showed that both types of pollution-free and polluted soils had loam-clay loam textures. Findings showed that the total amount of nitrogen in the pollution-free and oil-polluted soils in terms of percent were respectively 0.1 and 0.24. Considering to the optimum limits of nitrogen in soil (more than 2%), for agricultural purposes, it was it was determined that both samples of soil needed fertilizers containing nitrogen throughout the experiment in order to supply the plants with required nitrogen. Therefore, Hoagland Nutrient Solution was used once a week. The findings demonstrated that the amount of phosphor in both pollution-free and oil-polluted samples of soil was in ideal conditions considering the optimum limits of phosphor in soil (15 ppm). The collection of the results obtained from measuring the physicochemical features of both pollution-free and oil-polluted soils shows that both soils are of great similarities respecting these features particularly in texture, electrical conduction, and pH. Therefore, we can take the advantage of the mixture of these two kinds of soil for preparing different treatments of pollution, varying from 10% to 50% and examining growth of plants and the changes in the oil compounds of the samples in which the soils were mixed together with different percentages (from 10% to 50%).

Table 2.
Proportions of contaminated soil to polluted-free one in preparation of different treatments

Treatment type	Weight of polluted soil (g)	Weight of Pollution-free soil (g)
10%	10	90
20%	20	80
30 %	30	70
40%	40	60
50%	50	50
Control treatment without pollution	0	100

Preparation of different treatments of polluted soil

In this study, 5 different types of treatment were used in order to evaluate phytoremediation capacity of the species '*Polypogon monspeliensis'.* To do this, the pollution-free soil and the one polluted with petroleum were mixed together in 5 different types of treatment with 5 different weight ratios (Table 2). The weight ratios of polluted soil to pollution-free soil in these treatments were respectively 1 to 9 (treatment 10%), 2 to 8 (treatment 20%), 3 to 7 (treatment 30%), 4 to 6 (treatment 40%), and 5 to 5 (treatment, 50%). Having fully mixed the contaminated soil with pollution-free one with the different weight ratios mentioned above, we allowed this treatment to remain in open air to let them mixed together thoroughly and even further. Three weeks later, to make sure of homogenization of treatments, the soils were sifted through a sieve with 2 millimeter meshes. Afterwards, from each treatment 100 g soil was weighed by a scale with 0.001 error of measurement and put into 100 g plastic pots 9 cm × 9 cm insize (diameter × height).

Stages of growing plants and their transfer to different treatments of pollution

First of all, the seeds of the plants were sterilized with distilled water for 30 minutes. Then, they were washed with deionized water and were put between two layers of filter paper No. 4, and kept in the dark for 24 hours. In order to maintain humidity, the papers were moistened with water twice a day. The temperature of the greenhouse was kept constant between 20 up to 30 degrees centigrade. The quality of light in the greenhouse was considered 16 hours of light and 8 hours' dark. One week later, we witnessed germination of the seeds in all different species of the plant. Fourteen (14) days after being placed between the two layers of filter paper and growing to the size of 1 or 2 centimeters, the plants were transferred into 5 kg plastic pots with the dimensions of 21.5 cm × 21.5 cm (height × diameter), containing pollution-free soil so that they would grow in agricultural soil for one month without interference of pollution. Irrigation of pots was carried out with tap water once every two days. The one-month-old seedlings were taken out of the 5 kg pots filled with pollution-free soil and the roots were washed with deionized water. Afterwards, 2 seedlings were transplanted in each of the pots containing different treatments mentioned above. Three replications were considered for every treatment. In addition, a control treatment containing only pollution-free soil was considered in three replications in order to compare physiological features of the plants in the polluted treatments with the ones in control treatment without pollution. Also, in three replications we used the 5 different treatments of pollution mentioned above, without the presence of the plant, as control samples in order to compare the reduction rate of petroleum in both the treatments containing the plant and the ones without the presence of the plant. All stages of seedling and growth before and after transplanting in different types of treatment were carried out in the Greenhouse of Agricultural Research Station of Islamic Azad University, Saveh Branch. Geographical features of the research location were 35 degrees and 2 minutes north and 50 degrees and 34 minutes east, at an elevation of 960 meters above the sea level. Because of the vicinity with desert and low elevation, Saveh has a dry and hot climate. The average of day and night temperature in the greenhouse was kept constant between 22 to 31 degrees centigrade throughout the experiment period. Irrigation of the pots under experiment was done on alternate days and to the extent that it would not drain out of the bottom of pots. Due to the plant requirement to minerals, once a week, Hoagland Nutrient Solution (Epstein, 1972) was used to meet the nutritional needs of the plants.

Harvesting plants

Forty-five (45) days after transplanting the plants in different treatments polluted with petroleum, they were taken out of the oil-polluted soil as well as the pollution-free one in order to evaluate not only the changes occurred in the rhizosphere, but also the effects of pollution on indices of plant growth and the plant capacity for phytoremediation of soils contaminated with petroleum. Roots were gently removed from the soil and later they were washed with distilled water along with some part of the shoot. Afterwards, they were placed on filter paper to eliminate the excess water. After separation of the root from the shoot and measuring growth parameters of the root and shoot, the roots were placed inside an aluminum foil before they were put inside a refrigerator with temperature set at 4 °C ± 2 °C in order to evaluate the absorption rate of oil compounds by the plant. In addition, in order to examine the changes in the amount of oil compounds in the soil, the polluted soil containing plants and the one without plant were collected after the harvest and were transferred into a refrigerator with a temperature of 4 °C ± 2 °C.

Evaluation of shoot height and root dry and fresh weight parameters

Having taken the plants out of the soil, the shoot height in different treatments was measured by means of a ruler. The measurement of the shoot height was done from the collar up to the apical bud. To measure the dry and fresh weight, first the plants were removed out of the soil by cumulus and roots and shoots were washed with deionized water several times and then the excess water was eliminated from the plants by means of filter paper. The fresh weight was measured using a digital scale (Startorius, model CPA4235, Germany, accurate to 0.001). Later, each root was wrapped into an aluminum foil and transferred into an oven with the temperature of 70 degrees centigrade (Iran Behdad Gostar Co.) for 24 hours in order to calculate the dry weight. After taking them out of the oven, the dry weight was measured with the scale.

Measuring the rate of total oil and grease (TOG %)

Before transferring one-month-old seedlings into each of different treatments of pollution, the percentage of petroleum hydrocarbons existing in the soil of both different treatments of pollution and fully-polluted soil were tested according to 9071B and 1664A methods of America Environmental Protection Agency (EPA). In addition, in order to determine the effects of plants on reduction of total oil and grease in different treatments at the end of the day 45 after plantation, the reduction rate of petroleum hydrocarbons in the soil were specified. For this purpose, the plants were taken out of the pots containing the polluted soil, 45 days after plantation in different treatments with and without the plants, and later the samples of the soil belonging to the different treatments of pollution and also the polluted treatments without the plant were collected with a metal spatula and were stored inside glass containers. Having fastened their lids, they were transferred into a refrigerator with temperature of 4 °C ± 2 °C, which were kept there until the extraction with a Soxehlet. Before extraction, the soils under experiment were put in clean mortars which had been already washed with n-hexane, and then they were placed in an oven with temperature of 50 degrees centigrade for 3 hours in order to evaporate the existing moisture. After the samples were taken out of the oven, they were crushed inside a mortar which had been already washed with n-hexane and external objects like wood, leaves, and rocks were removed. In 3 replications, on Wattman No. 42 filter paper, we weighed an amount of 15 grams from each of the polluted treatments containing the plant and no-plant samples after the experiment and the fully-polluted prepared before the beginning of the experiment. Having folded the papers into the special filters of Soxehlet, which were free from any oil and grease, they were transferred into Soxehlet. Just before the beginning of the extraction, we washed the special beakers of Soxehlet with Methanol. Then we took the beakers out of the oven and removed the moisture on the beakers with clean cotton, and after cooling, we put on plastic gloves in order to prevent from leaving any fingerprints on the beakers. We added a few anti-explosion welding granules to each of the beakers and immediately measured their weight as primary weight with a scale. Later, 60 ml of n-hexane solvent was added to each beaker and the beakers were placed on a heater. The temperature program of the heater was adjusted according to the heating plate temperature of n-hexane solvent, i.e. 130 degrees centigrade. Afterwards, the filters containing different treatments were put into the beakers containing n-hexane solvent. The stages of working with Soxehlet involved 3 steps of immersion, elution, and solvent recovery. After the solvent recovery step ended, the beakers were gently picked up off the heater by means of pincers and they were moved from side to side for a few seconds so that the solvent vapors were eliminated thoroughly. For a full sedimentation, we added 1 gram sodium sulfate salt into each beaker and later we cooled and dried them inside a desiccator for 30 minutes and weighed them right away as the final weight.

To determine the percentage of the TOG present in the soil, first the primary weight of the beakers as well as the added one-gram sodium sulfate were subtracted from the final weight of the beakers. Then we expressed the calculated weight in the amount of the soil used in the experiment (15 grams) in terms of percent. In this way, the percentage of TOG inside each of the pollution treatments containing the plant and the ones without it was calculated before and after the experiment. Statistical studies were carried out by means of SPSS software, version 18, at the probability level of 95%.

Results

Results obtained from measuring the rate of total oil and grease in the fully-polluted treatment showed that the soil collected from the area contaminated with oil compounds, in average contained 10.18 percent of TOG with respect to the dry weight of the soil. The findings from comparison of different treatments containing the plant with the control without plants show that all treatments containing the plant species had a significant reduction at the probability level of 5% comparing to the control treatments without the plant after a period of 45 days. The maximum amount of reduction, as much as 84.85 percent (Fig. I) was related to the treatment 10% containing the plant and the minimum amount of reduction, i.e.7.57 percent, was related to the control treatment 50% without the plant. The results showed that as the petroleum concentration increased, its reduction rate decreased in the plant rhizosphere in a way that the minimum amount of petroleum reduction (22.42%) in the plant rhizosphere was related to the treatment 50% (Fig. I).

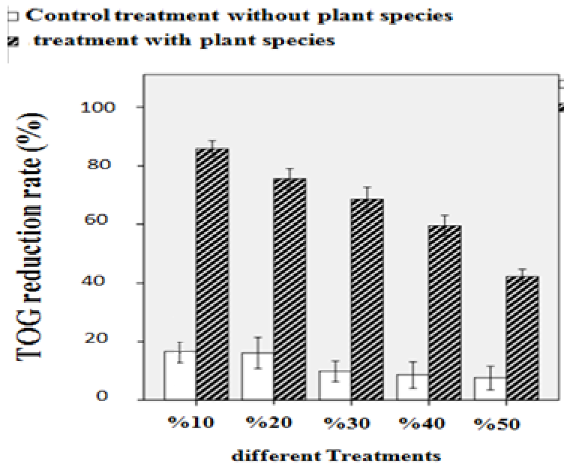

Fig. I. Reduction rate of TOG in different treatments of pollution planted with the species *Polypogon monspeliensis* and control treatment without plant

Our findings showed that the control treatment without pollution was significantly different from the treatment 50% only in the plant height which was higher. Fig. (II) shows that in the treatments 10% and 20% the plant height increased compared to the treatment without pollution, which probably is because the plant has used the oil compounds as well for further growth, or that is because the activity of most rhizosphere microorganisms of the plant in the treatments has led to the improvement in the growth of the plant height comparing to the treatment without pollution. In the treatments 30%, 40%, and 50%, the plant height decreased comparing to the treatment without pollution, showing that the plant is sensitive to higher density of oil pollution, i.e., it was not able to tolerate the pollution of 30% or more. The maximum growth in the plant height was related to the treatment 20% as much as 29.13 centimeters while the minimum growth was related to the treatment 50% that was 19.40 centimeters. Comparing the fresh weight of the control treatment without pollution with that in all other treatments showed a significant difference at the probability level of $p \leq 0.05$. Treatments of 10%, 20%, and 30% showed a significant increase in the fresh weight comparing to the control treatment without pollution. The findings showed that the plants in the treatments 40% and more had a significant decrease in the fresh weight of the root tissues. In addition, Results showed that there is no significant difference in the fresh weight of plants between treatments 10% and 30%.

Fig. (III) demonstrates the maximum amount of fresh weight was related to the treatment 20% as much as 0.058 gram whereas the minimum was related to the treatment 50%, i.e., an amount of 0.014 gram. Furthermore, the results showed that the difference between the dry weight of control treatment without pollution and the ones in the treatments of 20%, 30%, and 40% at the probability level of 5% was not significant while the difference was significant comparing to the other treatments. The treatment 10% had more dry weight comparing to the control treatment without pollution. There was no significant difference in the dry weights between treatments of 10% and 20% and treatments of 40% and 50%, and also between treatments of 40% and 50%. Fig. (IV) showed that the maximum amount of dry weight as much as 0.003 gram was related to the treatment 10% while the minimum was related to the treatment 50%, i.e., less than 0.001 gram.

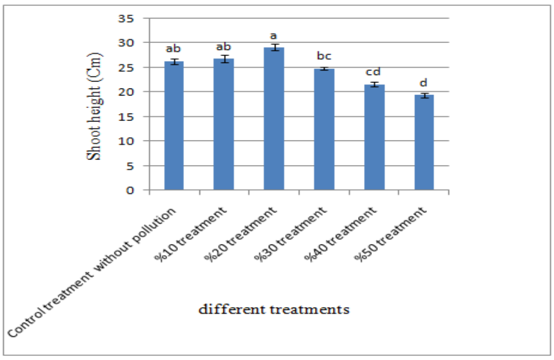

Fig. II. Comparison of shoot height of the species *Polypogon monspeliensis* planted in different treatments of pollution with control treatments without plant

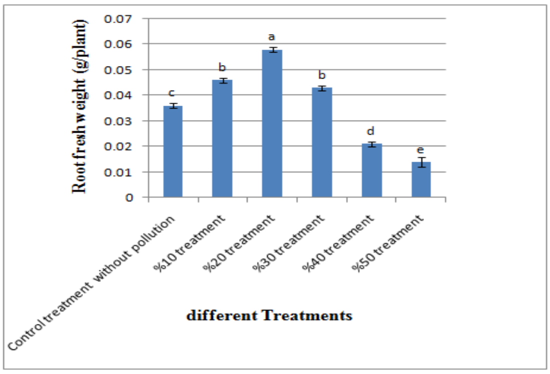

Fig. III. Comparison of root fresh weight of the species *Polypogon monspeliensis* planted in different treatments of pollution with control treatment without plant

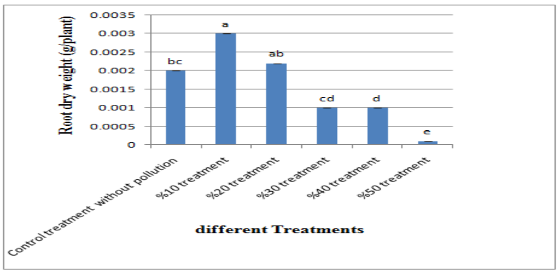

Fig. IV. Comparison of root dry weight of species *Polypogon monspeliensis* planted in different treatments of pollution with control treatment without plant

Discussion

Findings showed that both types of the soil, i.e., the polluted and the pollution-free soils, have a loam-clay loam texture. In addition, both soils are classified in group B of soil salinity devised by USDA and the salinity rate in both soils was between 0.13 up to 0.26 gram. The pH in both types of the soil was normal. The results demonstrated that the total amounts of nitrogen in the samples of pollution-free and oil-polluted soils in terms of percent were 0.1 and 0.24, respectively. Considering the optimum limits of total nitrogen in the soil (more than 0.2 percent), it was determined that for agricultural exploitation purposes both samples of the soil required fertilizers conaining nitrogen during the experiment to meet the needs of the plant for this nutrient. For this reason, during the experiment Hoagland nutrient solution was utilized once a week. Findings showed that the amount of organic carbons (%OC) in both samples of pollution-free and oil-polluted soils was in ideal conditions. The amount of phosphor in both samples of pollution-free and oil-polluted soils was in good conditions considering the optimum limits of phosphor in soil (15 ppm). The results respecting the measurement of potassium rate showed considering the optimum limits of potassium in soil (350ppm), its rare was ideal in the samples of pollution-free soil, but the polluted sample suffered from shortage of potassium. Amount of sodium in the samples of pollution-free and oil-polluted soils was 92 milligrams per kilogram (ppm). Measuring the rate of calcium and magnesium showed that the amount of calcium in pollution-free and oil-polluted samples were respectively 7 and 6 milliequivalents per liter (meq/l). The amount of magnesium in pollution-free and oil-polluted samples were respectively 10.1 and 2.19 milli equivalents per liter (meq/l). Amount of iron in pollution-free and oil-polluted samples were respectively 9.18 and 6.83 milligrams per kilogram (ppm). Also, the amount of zinc in pollution-free and oil-polluted samples were respectively 2.86 and 15.3 milligrams per kilogram (ppm). The amount of copper in pollution-free and oil-polluted samples were respectively 1.28 and 8.6 milligrams per kilogram (ppm). Amount of manganese in pollution-free and oil-polluted samples were respectively 6.68 and 4.6 milligrams per kilogram (ppm). Putting the results obtained from measuring the physicochemical features of pollution-free and oil-polluted samples of soil together showed that both types of soil were much alike respecting these features especially in parameters of texture, electrical conduction, and pH. Therefore, we were able to take the advantage of the mixture of these two types of soil for preparing different treatments of pollution, i.e., from 10% up to 50% and evaluate the growth of plants and the changes taken place in oil compounds of those treatments (10% to 50%).

Phytoremediation is the use of plants and microorganisms for elimination or detoxification of environmental pollutants. Phytoremediation has drawn a lot of attention towards itself in recent years because of easy application, functionality in the very same polluted location as well as many other advantages (Merkel et al., 2004). Phytoremediation of the soil polluted with petroleum is mainly based on the operation of microorganisms present in the plant rhizosphere (Frick et al., 1999). Therefore, the root system in the meantime improves the performance of microorganisms in elimination of pollution by supplying oxygen, growth factors, some enzymes, and hormones. Our findings have shown that vegetation can cause a decrease in soil pollution with oil compounds (Cunningham et al., 1999) and plants from legumes family such as *'Calopogon mucunoides, Centrosema brazilianum, Styllosanthes Capitata*, and also plants from *Graminace* family like *Cyperus aggregates, Brachiria brizantha*, and *Eleusine indica* have demonstrated remarkable capacity in reducing oil pollutions (Merkel et al., 2004). The results we obtained showed that in all the different treatments, i.e. from 10% up to 50% used during the period of 45-day experiment, the treatments containing the plant were of a considerable difference in reduction of TOG rate in the soil, i.e. the amount of TOG reduction was more in those treatments. Results showed that as the density of TOG increases, its reduction in different treatments decreased probably because of increasing phyto-toxicity as well as toxification of rhizosphere microorganisms in higher TOG concentrations. However, the plants have been able to reduce TOG in different treatments. Comparison of the treatments containing the plant species with the ones without pollution after 45 days shows that the microorganisms existing in the plant rhizosphere play a major role in the process of removing oil compounds, and the plants play some sort of supportive role towards microorganism activities. Our findings conform to the results of Agamuthu and the colleagues (2010) in which they introduced rhizodegradiation as the main mechanism of elimination of oil compounds in the rhizosphere of the plant *Jatropha Curcas*. The reaction of plants to different oil concentrations was examined during the period of 45-day experiment. No plant deaths were observed in any of the treatments, i.e. from 10% up to 50%, at the end of this period. However, symptoms of plant toxification such as yellowing leaves and decrease in growth were seen compared to the control treatments. These symptoms were in accordance with findings of Vouillamoz and Mike (2009). The plants in the treatments with higher density of pollution, i.e. more than 20%, showed clear signs of toxification after 45 days. Physical effects of petroleum on the plants include covering some parts of plant shoots, and as a result, they creates a block against the sun rays necessary for photosynthesis. In addition, by covering the soil surface, it prevents from full ventilation of the soil, as well (Aniggboro and Tonukari, 2008). Also, the chemical effects of oil on the plants include gradual disorders in metabolism, growth, and development of plants. Petroleum causes decrease in growth indices of plants such as height, number of leaves, leaf area, chlorosis, dry and fresh weight of plants, root biomass (Omosun et al., 2008), deaths of leaf cells, reduction of photosynthesis pigments (Mallah et al., 1998), and slowing down the absorption of nutrients (Rosso et al., 2005). Also, some substances in the petroleum can dissolve the membrane and this leads to the destruction of the plant roots (Maila et al., 2002).

Our findings showed that there was an increase in the shoot height of this species comparing to the control treatment without pollution, which means that the rhizosphere microorganisms of this species in those treatments caused an increase in the absorption of minerals from the soil and as a result, this leads to the increase in shoot heights. Our findings demonstrated that the fresh weight of the species *Polypogon monspeliensis*, in all different treatments of pollution was considerably different from the fresh weight in control treatment without pollution. Treatments of 10% up to 30% showed a significant increase in the amount of the root fresh weight. Treatments of 40% and 50% had considerable reduction in the root fresh weight comparing to the control treatment without pollution. This means that as the concentration of oil compounds increases in the soil, i.e. more than 30%, the plant's tolerance towards it decreases. In addition, comparison of the root dry weight in this species showed that except for treatments of 20%, 30% and 40%, the root dry weight in other treatments was significantly different from the treatment without pollution. However, apart from treatments of 10% and 20%, the root dry weight decreased in the other treatments. This means that increase in the concentration of oil percentage in treatments of more than 20% leads to the reduction of substance synthesis in the root tissues.

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