RESEARCH PAPER

Laboratory investigation of the removal of total petroleum hydrocarbons from oil-contaminated soil using Santolina plant

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Abstract

Petroleum hydrocarbons are one of the most common groups of persistent organic pollutants in the environment. Plant remediation is a new technology in which resistant plants are used to remove or reduce the concentration of organic and mineral pollutants and dangerous compounds from the environment. This study aims to investigate the effect of the Santolina plant on the removal of total petroleum hydrocarbon (TPH) from oil-contaminated soil at different concentrations. First, the soil was collected from around the Ahvaz oil field and contaminated with oil concentrations of 5, 7, and 10% by weight. The amount of TPH was measured during 4 months with two repetitions and at five retention times. A GC device with a FID detector was used to determine the remaining TPH concentration. The results showed that 91% of the petroleum hydrocarbons in treatment with 5% oil pollution were removed by the Santolina plant in 120 days. The results of this study showed that the most changes in the microbial population in the 7% treatment were 9.78×10^6 CFU/g in 120 days. The results of this research showed that the Santolina plant has a high ability to remove oil pollution from contaminated soil.

Keywords: Bacteria, Oil pollution, Santolina, Soil, Total petroleum hydrocarbons

1. Introduction

Today, due to the large use of petroleum compounds, the pollution caused by these compounds is very widespread in the world.[1] Currently, more than 3,600,000 wells have been drilled by oil companies around the world. There are about 6000 oil production wells in America. The number of oil wells in Western Europe is ~6000 rings, and the number of known oil and gas basins in the world is more than 22,000 basins.[2] Ahvaz oil field also has 464 oil well rings. Soil acts as the main source of pollution in the world. Research on the use of plants and trees in cleaning soil contaminated with various organic and mineral compounds and heavy metals has become very important in the last few years.[3] Petroleum hydrocarbons are one of the most common groups of persistent organic pollutants in the environment. In a way, the accumulation of pollutants in the soil can have destructive effects on the environment and human health. Pollutants in the soil can enter the food chain and pose a serious threat to the health of animals and humans.[4] There are many physical and chemical methods to deal with oil pollution in the soil. Many of these methods are used less due to high costs and harmful side effects. Biological modification is a type of purification technology that uses the biological activity of living organisms to reduce the concentration or solubility of pollutants, including petroleum hydrocarbons.[5] The soil-plant system is particularly important in comparison with other complementary methods in places where

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enough land is available due to its high efficiency, low cost, and lack of specialized manpower. Therefore, in recent years, more attention has been paid to biological methods, such as phytoremediation.[6] Plant remediation is a new technology in which resistant plants are used to remove or reduce the concentration of organic and mineral pollutants and dangerous compounds from the environment. Plant bioremediation is a new, effective, and environmentally friendly technology that has been proven to remove many pollutants, especially petroleum compounds.[7] Bioremediation is an economical method for managing waste compounds, especially excess amounts of petroleum hydrocarbons, polycyclic aromatic hydrocarbons, explosives, organic compounds, and nutrients.[8] The waste compounds that can potentially be managed using bioremediation are very diverse. Plant bioremediation can be a powerful tool in oil waste management. The important point for the successful remediation of oil-contaminated soils by the plant remediation method is the selection of suitable plants with the ability to grow and adapt to the polluted environment.[9] These plants should be resistant to the existing pollution in the region and have maximum germination, growth, and development, as well as the special surface of the roots. Plants are able to stimulate and increase the activity of the microbial population that destroys oil pollutants by releasing nutrients and leaching various compounds, including organic acids and sugar compounds into soil and transferring oxygen to the root region.[10] In many cases, even the physical presence of the plant can improve soil conditions. The plant increases the strength of soil grains and improves soil structure, as well as changes in soil hydrology by increasing water holding capacity and preventing soil erosion.[11] Although the plant treatment of pollutants has a complicated mechanism. But it reduces available organic compounds. In general, annual grasses have been used to remove oil compounds in many studies.[12] In spite of the relatively extensive studies conducted in the field of bioremediation of soil contaminated with petroleum compounds in the world, very limited and scattered studies have been conducted in Iran. Researchers conducted a study on the growth of fescue plants in soil contaminated with different concentrations of crude oil (1–3–5–7–10%) for 120 days.[13] In this study, the reduction in oil pollution was reported as 7.36%. Another group of researchers conducted a study on the *Cyperus laxus* plant to remove total petroleum hydrocarbons (TPH). The results of this research showed that the *C. laxus* plant is able to grow in soils with a TPH concentration of up to 200 g/kg.[14] In this study, TPH concentration was reduced by 90% in laboratory conditions. The researchers conducted a study on the efficiency of removing TPH from the soil using the combined method of plant bioremediation and agricultural mechanical operations.[15] In this experiment, which was conducted on corn and elephant grass for 2 weeks, the concentration of total hydrocarbons decreased by 5.77 and 83%, respectively. Other researchers investigated the ability of five different plant species to remove petroleum compounds from highly polluted soils with TPH concentrations of more than 50,000 mg/kg. They found that a flax plant is able to remove 87.63% of TPH. Other researchers used sorghum plants to remove oil pollution, with a concentration of 58.2%. The results of this study showed that after three months, the rate of TPH reduction was 20%. Other researchers conducted a study on two types of Jagan plants for 360 days.[16] In another study, in concentrations of 5 and 10% oil pollution, the percentage of TPH reduction in the presence of a sunflower plant was 59.25%, and in the presence of a vetiver plant, it was reported to be 41%. Other researchers analyzed pumpkins for 18 weeks and at four concentrations of pollution. The results of this research showed that the percentage of TPH reduction in the concentration of 1.59% is equal to 38.94%.[17]

In the present study, the Santolina plant was used for plant treatment. Santolina has desirable morphological, physiological, and ecological characteristics for cultivation and development. The root system of Santolina is extensive and small. For this reason, it can grow very fast. The root of the Santolina plant makes this plant resistant to drought. In this study, the removal rate of TPHs from oil-contaminated soil was evaluated with the help of the Santolina plant for 4 months. The studied soil was collected from around the Ahvaz oil field wells and then contaminated with certain concentrations. In this study, parameters, pH, TPH, EC, organic solids, and population of heterotrophic bacteria of soil were evaluated for 4 months at three concentrations of 7, 5, and 10% oil pollution.

2. Materials and methods

2.1. The method of artificial contamination of soil

The soil collected from around the Ahvaz oil field was crushed well and spread in a thin layer. Then crude oil was dissolved in acetone with a ratio of 1–3 and added to the soil in the form of a spray. The pots were filled with 30 cm of contaminated soil with different concentrations. The soil weight of each pot was 7 kg, and it was prepared for planting. In this study, two types of treatments were prepared in three concentrations, including a treatment with plants with 5% contamination, a treatment without plants with 5% contamination (control treatment), a treatment with plants with 7% contamination, a treatment without plants with 7% contamination (control treatment). The treatment with plants had 10% contamination and the treatment without plants with 10% contamination (control treatment). Two replicates of each treatment were prepared. Due to two repetitions, 12 cases (three cases of treatment with plants and three cases of treatment without plants) have been performed on the samples for analysis.

2.2. Sampling operation and digestion of samples

This study was investigated five times (15–30–60–90–120 days). To carry out the analysis, some soil was taken from each of the pots, stored in a zip bag, and then transferred to the laboratory for analysis.

2.3. Total petroleum hydrocarbon assay

An agilent gas chromatograph equipped with an FID detector was used to measure the concentration of TPHs. The detection limit of the device was 1 ppm, and its recovery percentage was between 64 and 70%. The determination of the concentration of TPHs was done based on the United States Environmental Organization (UNEP) method. In order to determine the concentration of TPHs in the soil, the first 10 g of the sample were extracted in a Soxhlet extractor with 250 ml of a mixture of hexane and dichromate. Then, 1 μl of the obtained extract was measured and determined using an Agilent 6890Ng gas chromatograph equipped with an FID flame ionization detector and a B-5 capillary column.

2.4. Counting the number of heterotrophic bacteria

To determine the microbial population of the soil through different treatments, the Heterotrophy Bacteria Plate Counting method was used. A colony counter was used to count the bacterial colonies formed on the petri dishes after the incubation period. The microbial population was expressed as CFU/g.

2.5. The method of determining total volatile solids

Weighing was used to determine the total volatile solids of the soil sample based on the 1684 EPA method.

2.6. Data analysis method

SPSS software, version 17 (IBM, New York, USA) and analysis of variance statistical tests were used to check the difference of means in different treatments. In this research, the level of significance was considered as P value less than 0.05. Excel software was also used to draw the graph.

3. Results

The physical and chemical characteristics of the studied soil are presented in Table 1. In Table 1, it can be seen that the soil used has 50% porosity. Also, the texture of the soil is sandy loam, and the percentage of sand is 80.1%. The studied soil was contaminated with concentrations of 5, 7, and 10% by weight. The amount of soil TPH was determined at the beginning of the work. Table 2 shows the amount of TPH at the beginning of work.

3.1. The amount of total petroleum hydrocarbon removed during the plant treatment process

The amount of TPH removal changes in plant treatments and control treatments in different concentrations (5, 7, and 10%) were investigated. According to Table 3, the best removal efficiency of

Table 1. Analysis of the physical and chemical characteristics of the studied soil.

Characteristics	Unit	Measurement method	Value
pH	–	Soil slurry with a pH meter	7.1
EC	dS/m	Soil slurry with an EC meter	8.4
N	%	Kjeldahl	0.086
P	mg/kg	Olsen	3.6
Texture		Hydrometric	Sandy loamy
Clay	%	Hydrometric	8.9
Sand	%	Hydrometric	80.1
Silt	%	Hydrometric	10.9
Porosity	%	Apparent specific gravity	50
Mineral solids	%	Gravimetric	92.44
Organic solids	%	Gravimetric	7.6
Humidity	%	Gravimetric	2.015

Table 2. Measured concentrations of oil pollution in the investigated soil at the time of planting.

The percentage of pollution caused by (artificial) initial concentration	The amount of pollution measured at the beginning of working with the GC (mg/kg)
5	49,600.42
7	68,748.63
10	99,548.41

Table 3. The rate of changes in total petroleum hydrocarbon removal in the treatments with plants and controls during 4 months in terms of mg/kg.

Time	Herbal treatments			Control treatment		
	5%	7%	10%	5%	7%	10%
0	49,600.42	68,748.63	99,548.41	49,600.42	68,748.63	99,548.41
15	30,751.38	45,717.63	71,674.84	36,802.85	59,671.46	91,385.64
30	25,047.6	38,086.37	63,213.84	35,711.74	53,417.84	88,299.62
60	11,755.62	20,418.52	40,615.93	30,652.73	44,755.73	71,674.84
90	9542.7	17,338.62	30,481.74	21,575.63	38,705.63	67,692.73
120	4463.84	9349.73	19,611.73	19,343.83	35,061.73	63,014.74

TPH in all concentrations in 120 days was 91, 86.4, and 80.3%, respectively, in treatments with plants at concentrations of 5, 7, and 10% of oil pollution. Also, the lowest amount of TPH removal was observed in all concentrations of control treatments and 15 days. The percentage of TPH removal measured in concentrations of 5, 7, and 10% of oil pollution was 25.8, 17.5, and 8.2%, respectively.

3.2. pH changes during the plant treatment process

Changes in pH during the plant treatment process were measured in three concentrations (5, 7, and 10%) of oil pollution during 4 months. According to Table 4, it can be seen that the pH has increased during the treatment process. Also, the control treatments had more pH changes than the treatments with plants. The most changes in pH in all treatments were on day 120, and the most changes in three concentrations of 5, 7, and 10% of oil pollution in the control treatments were 9.68, 10.33, and 9.8%, respectively.

3.3. EC changes during the plant treatment process

In this study, EC changes were measured during 4 months. During the plant process, the amount of EC has decreased. The reduction of EC in treatments with plants was more than in the control treatments. According to Table 5, it can be seen that the most EC changes of treatments with plants in concentrations of 5, 7, and 10% were 2.63, 2.76, and 2.8 ds/m, respectively, on day 120. In the treatment with plants and 10% of oil pollution, the highest percent reduction in EC has been observed and reached 74.31%.

Table 4. The amount of pH changes in treatments with plants and control treatments during 4 months.

Time	Herbal treatments			Control treatment		
	5%	7%	10%	5%	7%	10%
0	6.4	6.48	6.63	6.4	6.48	6.63
15	6.46	6.56	6.73	6.46	6.56	6.73
30	6.42	6.74	6.89	6.68	6.89	6.92
60	6.64	6.82	6.92	6.82	6.97	7.09
90	6.81	6.89	6.96	6.98	7.10	7.15
120	6.88	6.95	7.02	7.02	7.15	7.28

Table 5. The amount of EC changes in the treatments with plants and controls during 4 months in terms of dS/m.

Time	Herbal treatments			Control treatment		
	5%	7%	10%	5%	7%	10%
0	9.91	10.49	10.9	9.91	10.49	10.90
15	6.46	6.51	6.70	9.95	10.21	10.61
30	3.49	3.68	3.50	8.53	8.87	9.31
60	2.98	3.10	3.42	6.27	7.71	8.06
90	2.79	2.94	2.97	5.73	6.24	7.81
120	2.61	2.73	2.76	5.01	6.12	7.41

3.4. Population changes of microbial activity during the plant treatment process

The amount of heterotrophic soil microbial population was counted on the first day and the 120th day. During 120 days, the amount of soil microbial population increased, and this increase was greater in the treatments with plants than in the control treatments. According to Table 6, the amount of heterotrophic soil microbial population in concentrations of 5, 7, and 10% oil pollution in treatments with plants is equal to $9.64 \times 10^6, 9.78 \times 10^6$ *and* 8.31×10^6 CFU/g, respectively. The laboratory results show that the 7% oil pollution treatment had the highest increase in the microbial population.

3.5. The amount of changes in organic solids during the plant treatment process

The amount of changes in organic solids was measured during 4 months of plant treatment. The highest percentage reduction in all concentrations was observed in 120 days and in the treatments with plants, which can be seen in Table 7. In the concentration of 5, 7, and 10% of oil pollution, the percentage reduction measured was 65.49, 66.40, and 66.41%, respectively. The treatment with 10% of oil pollution had the highest reduction in total solids.

Table 6. The amount of changes in the number of primary and secondary heterotrophic bacteria in treatments with plants and controls in terms of CFU/g.

Time	Herbal treatments			Control treatment		
	5%	7%	10%	5%	7%	10%
0	2.86×10^6	2.91×10^6	2.37×10^6	2.86×10^6	2.91×10^6	2.37×10^6
120	9.63×10^6	9.78×10^6	8.32×10^6	8.10×10^6	8.19×10^6	6.95×10^6

Table 7. Percentage changes of organic solids in the treatments with plants and controls during 4 months.

Time	Herbal treatments			Control treatment		
	5%	7%	10%	5%	7%	10%
0	14.75	15.30	16.02	14.75	15.30	16.02
15	13.91	14.95	15.97	12.94	13.11	13.22
30	11.96	12.22	12.55	12.05	12.31	12.44
60	7.65	7.74	9.55	9.81	10.78	11.78
90	7.20	7.02	8.00	9.03	9.25	10.12
120	5.08	5.15	5.37	8.47	8.78	8.95

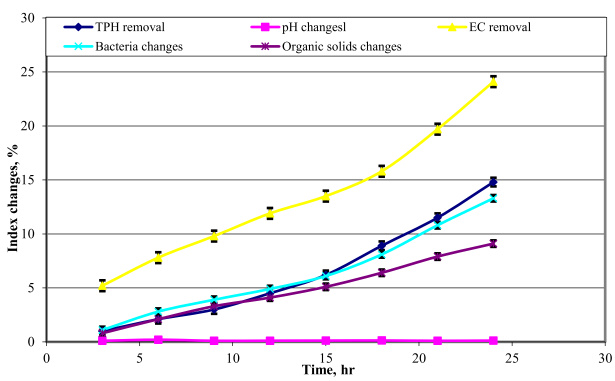

Fig. 1. Process index changes in an operational day.

3.6. Evaluation of changes in indicators on an operating day

Figure 1 shows the changes in process indicators in one operational day. As can be seen in Fig. 1, the changes in the removal of petroleum hydrocarbons, electrical conductivity, and changes in pH are presented. In Fig. 1, the changes in primary and secondary bacteria are also presented. Fig. 1 also shows the changes in organic solids in one operational day.

4. Discussion

The efficiency of the plant treatment process, like most biological processes, depends on several environmental factors. The most important of these factors are organic compounds, toxic compounds, humidity, temperature, ion exchange capacity, type of soil texture, and pH. However, there are many and sometimes unknown factors that may affect the efficiency of the process of plant treatment. In this study, it was tried to maintain the same environmental conditions, such as light, temperature, and humidity, for all treatments. According to the results, the higher microbial population and also the removal of TPH in the treatments cultivated with plants compared to the control treatments can be due to the multiplication of plant roots and the provision of a growth matrix. It can also be due to the increase in plant root secretions and the result of stimulating the microbial population that decomposes petroleum hydrocarbons. By secreting organic compounds such as glucose, enzymes, and complex carbohydrates, plant roots provide a suitable source of carbon and energy for microorganisms in the root region. The root secretions cause loosening of the soil, and as a result, oxygen is transferred to the deeper parts of the soil, which improves the growth of plants and pollutant-decomposing microorganisms. Researchers researched sunflower and vetiver grass to remove oil hydrocarbons in two concentrations of 5 and 10% of oil pollution. In this study, the percentage of TPH reduction in sunflower was 25.59% and in vetiver was 41%.[11] Other researchers conducted a study on pumpkins for 18 weeks and at four concentrations of oil pollution. In this study, the highest percentage of TPH reduction was 94.38%.[12] Other researchers used a vetiver plant to remediate soil contaminated with trichloroethylene. The highest percentage of reduction of trichloroethylene in contamination with a ratio of 1 : 1, equal to 71%, and the highest reduction of trichloroethylene in contamination with a ratio of 1 : 3, equal to 41% was obtained.[13] Other researchers investigated the ability of five different plant species to remove petroleum compounds from highly polluted soils with TPH concentrations of more than 50,000 mg/kg. The results showed that the flax plant had the highest TPH removal percentage of 87.63%.[14]

In the present study, the best TPH removal efficiency was 91% in 5% concentration of oil pollution and 120 days. The lowest percentage of TPH removal related to control treatment, with 10% oil pollution in 15 days, was 8.2%. The treatments of 5, 7, and 10% oil pollution had a positive correlation with time at the level of 0.01. According to the results obtained at concentrations of 7 and 10% of oil pollution, the TPH removal efficiency has been reduced by 5% of oil pollution. This is due to the presence of high pollution and inhibiting the growth of plants and bacteria. However, in all the treatments with the plant in all concentrations of oil pollution, the amount of TPH removal was higher than in the control samples, which shows that the Santolina plant can remediate oil-contaminated soils. Also, this study shows a significant difference ($P < 0.05$) between treatments with plants and control of the amount of TPH removal in 4 months. The greatest reduction of organic solids was related to the treatment with plants, and 10% of the contamination in 120 days was 66.41%. Therefore, it can be concluded that organic solids are used as a carbon source for microorganisms in the soil rhizosphere.

The studies showed that the 5 and 7% treatments on days 60, 90, and 120 had a significant difference ($P < 0.05$) with the control, and the 10% treatment on the 90th and 120th days had a significant difference ($P < 0.05$) with the control. The results of this study showed that the increase in the number of heterotrophic bacteria in the 120 days was due to the fact that the organic matter in the soil, along with hydrocarbons, was considered a food source for the bacteria. Also, the increase in the number of heterotrophic bacteria in the treatments with plants was more than in the control treatments. It shows that the number of heterotrophic bacteria has decreased in treatments with 10% oil contamination compared to other treatments. This is due to the presence of high pollution and the creation of an inhibitory state for the growth of bacteria. Also, the studies show that all treatments on the 15th and 120th days of each other had a significant difference ($P < 0.05$, and in 120 days, they also had a significant difference with the control sample).

Microorganisms and plants need minerals and nutrients. With the growth of microorganisms and the increase in their number, the consumption of mineral substances in the soil increases, and therefore, EC decreases. EC is an indicator of the amount of minerals in the soil. With the increase in the consumption of plants and bacteria, the mineral content of the soil decreases. The results showed that all treatments had a positive correlation with time at the 0.01 level. Also, the studies show that there is no significant difference between any of the treatments and days in the main treatment and the control. According to the results obtained in all treatments with plants at all concentrations of pollution, the removal rate of TPH was higher than in the control samples. This shows that the Santolina plant can survive and remediate oil-contaminated soil due to the existing pollution. Organic compounds in the soil, along with hydrocarbons, have been a source of food for bacteria. As a result, the amount of organic solids in the soil has decreased during the plant treatment process. Also, the number of heterotrophic bacteria in the soil treated with plants during the plant remediation process was more than that of the control treatments.

5. Conclusion

In the present study, the best TPH removal efficiency was 91% in 5% concentration of oil pollution and 120 days. The lowest percentage of TPH removal related to control treatment, with 10% oil pollution, and in 15 days, was 8.2%. The treatments of 5, 7, and 10% oil pollution had a positive correlation with time at the level of 0.01. According to the results obtained at concentrations of 7 and 10% of oil pollution, the TPH removal efficiency has been reduced by 5% of oil pollution. Results show that the greatest reduction of organic solids was related to the treatment with plants and 10% contamination in 120 days, which was 66.41%. This study shows that all treatments on the 15th and 120th days of each other had a significant difference ($P < 0.05$, and in 120 days, they also had a significant difference between the control sample). The present work shows with the growth of microorganisms and the increase in their number, the consumption of mineral substances in the soil increases and, therefore, EC decreases. The results showed that all treatments had a positive correlation with time at the 0.01 level. Also, the studies show that there is no significant difference between any of the treatments and days in the main treatment and the control. This work shows that the Santolina plant can survive and remediate oil-contaminated soil due to the existing pollution.

Author contribution

Hadi Tarighat: software, data curation, reviewing, and editing. Peyman Boustani: visualization, investigation, and validation. Farshad Farahbod: supervision, writing-original draft preparation, conceptualization, and methodology.

Conflicts of interest

There is no conflict of interest.

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