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Evaluation of the Growth and Phytoremediation Ability of Ornamental Tall Fescue and Sunflower in Contaminated Soils to Petroleum Hydrocarbons

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Abstract

Petroleum sludge is the waste of crude oil refining processes, and are the most priority organic pollutants found in the environment. The present study was carried out to evaluate the growth and phytoremediation ability of ornamental tall fescue (*Festuca arundinacea* Schreb.) and sunflower (*Helianthus annuus* L.) plant to petroleum sludge produced by Isfahan Refinery in contaminated soils in a greenhouse experiment. For this purpose, the air-dried petroleum sludge sample was added to an uncontaminated soil at ratios of 0 (control), 10 and 20% (w/w) and mixed thoroughly. Plastic pots were filled with called mixture (3 kg). The experiment layout consisted of split-plots arranged in a completely randomized design with three replicates. The results showed that with increasing sludge level from 0 to 10 and 20 %, the tall fescue germination decreased significantly ($P<0.01$). In sunflower plant this decrease was not significant. The mean values of plant heights and root and shoot dry weights for tall fescue only in 20% level of petroleum sludge showed a significant reduction when compared with control. However, in sunflower plant, this reduction was not significant in all three petroleum sludge levels ($P<0.01$). The highest reduction of total petroleum hydrocarbons (89%) in tall fescue rhizosphere was observed in petroleum sludge level of 10%. By increasing the petroleum sludge up than 10%, total petroleum hydrocarbons in rhizosphere of two plants significantly decreased ($P<0.01$). Because, tall fescue plant produces more roots than shoots and have more fibrous root systems that caused more reduct TPH, application this plant recommend as a appropriate species for phytoremediation of contaminated soils to petroleum sludge of Isfahan Refinery.

Keywords: Isfahan oil refinery, Petroleum sluge, Shoots and roots yield, Total petroleum hydrocarbons (TPHs).

INTRODUCTION

These compound account for 65 to 95% of crude oils and are the most priority organic pollutants (POPs) found in the environment (Xiao *et al.,* 2015). The contamination of soils to PAHs has received world wide attention due to their persistence, its high toxicity, mutagenicity, carcinogenicity, and genetic toxicity and can be caused extensive concerns (Zheng *et al*., 2021; Dupuy *et al*., 2021). In recent decades, phytoremediation has been considerate as one of the cheapest and economical solutions with continual effect for decreasing aromatic hydrocarbon contaminations from contaminated soil (Nguemte *et al*., 2018). To obtaining success in this filed, it is necessary to choose plants which are adaptable and has the capacity of grow in contaminated environment (Wei and Pan, 2010). Previews studies done by different researchers showed that some of common species used in the phytoremediation of PAH compounds of contaminated soils include tall fescue (*Festuca arundinacea*) (Liu *et al*., 2010), ryegrass (*Lolium perenne*) (Geo *et al*., 2017; Cheema *et al*., 2010), alfalfa (*Medicago sativa*) (Wei and Pan, 2010), Sorghum *(Sorghum bicolor), (*Banks *et al*., 2003b); maize (*Zea mays*) (Dupuy *et al*., 2021); five ornamental plant species)Xiao *et al*., 2015); *Tagetes patula* L. (Sun *et al*., 2011); smooth meadowgrass (*Poa pretensis*) (Palmroth *et al*., 2006), Weed plant (Qixing *et al*., 2011), crabgrass (*Digitaria sanguinalis* (L.) Scop.) (Klomjek and Nitisoravut, 2005), bermudagrass (*Cynodon dactylon* (L.) Pers) (White *et al*., 2006), tall fescue (*Festuca arundinacea*) and switchgrass (*Panicum virgatum* L.) (Chen *et al*., 2004). These plants in consensus with microorganisms can increase decomposition of aromatic hydrocarbonic compounds of contaminated soils (Xiao *et al*., 2015).

The use of ornamental plants (OPs) in the field of phytoremediation is scarcely reported. The use of OPs can simultaneously remove contaminants and bring improvement in aesthetics of the site. The biomass of OPs produced after such activities can be used and sold as pot plants, cut flowers, essential oils, perfumes, air freshener's production and metal phytomining. The OPs also present a lower risk of HMs bioaccumulation compared to crop plants (Ali Khan *et al*., 2021). Merk *et al*. (2005) found that cultivation of *Centrosema brasilianum* (L.) Benth and *Urochloa brizantha* in soil which contaminated by petroleum components can improve soils by root secretion, aeration and setting soil water. Chen *et al*. (2004) used ^{14}C-labeled pyrene to investigate the fate of pyrene in the rhizosphere of tall fescue (*Festuca arundinacea*) and switchgrass (*Panicum virgatum* L.) and found that after 190 days of incubation, 37.7% and 30.4% of ^{14}C-pyrene was mineralized in soil planted with *F. arundinacea* and *P. virgatum* L., respectively, compared with only 4.3% mineralization was observed for the unplanted control. Adewole *et al*. (2010) performed a field plot trial to investigate the remediating ability of two sunflower species (*Helianthus annuus* and *Tithonia diversifolia*) on soil polluted with effluents from the paint industry, and after in situ second successive cultivation, the removal rates of heavy metals by *H. annuus* and *T. diversifolia* were Cu 21.1% and 9.8%, Pb 15.7% and 26.3%; and Cd 44.1% and 7.8%, respectively.

The results of Ravanbakhsh *et al*. (2009) research's showed, petroleum hydrocarbon contamination reduced the growth of the surveyed plants significantly. Sorghum and common flax reduced TPHs concentration by 9500 and 18500 mg kg^{-1}, respectively, compared with the control treatment. Askary *et al*. (2012) were reported ornamental trees can be used as bioaccumulator in low concentrations of petroleum pollution. Iraji Asiyaabadi *et al.* (2015) reported, the reduction of the concentration of petroleum hydrocarbons in the contaminated soil cultivated with *Sorghum bicolor* and *Hordeum vulgare* was 52 and 64%, respectively, which was 30% more than the contaminated soil without plants. The results of Kaimi *et al.* (2015)

research's in Japanese environmental conditions indicated that eight plant species (Italian ryegrass, sorghum, maize, alfalfa, Bermuda grass, rice, kudzu and beggar ticks) caused a more significant decrease in the TPH concentration in the planted diesel-contaminated soil than in the unplanted soil. Xiao *et al.* (2015) in pot-culture experiments of China city were reported, that removal rates of PAH by two plant fire phoenix and *Medicago sativa* Linn. were significantly higher than those of other tested ornamental plants. So that, fire Phoenix and *Medicago sativa* Linn. with removal rates of 99.40% and 98.11%, respectively, are excellent ornamental plant species with great potential for removing PAHs in contaminated soils than those of other tested ornamental plants. The results researchs Nguemte *et al.* (2018) in assess the phytoremediation potential of 6 plant species in four Cameroonian cities showed, only 3 plants (*Eleusine indica*, *Cynodon dactylon*, *Alternanthera sessilis*) survived and developed throughout the 150 days of experimentation. Total petroleum hydrocarbon concentration in soils have been reduced to more than 80% for *E. indica* and *C. dactylon,* to 77% for *A. sessilis* and 57% in non-planted soil.

Yuanyuan *et al.* (2020) in evaluate the phytoremediation efficiency and rhizosphere regulation mechanism of fire Phoenix (a mixture of *Festuca* L.) in polycyclic aromatic hydrocarbon (PAH) contaminated soils reported, after 150 days of planting, the removal rates of the total PAHs reached 64.57% in contaminated soils with low-PAH (104.79–144.87 mg kg^{-1}) and 68.29% in contaminated soils with high-PAH (169.17–197.44 mg kg^{-1}). Results researchs Wang *et al.* (2022) showed that fire Phoenix could grow in soil contaminated by high and low concentrations of PAHs. After being planted for 150 days, the total petrolieum hydrocabon removal rate in the high and low PAH concentrations was 80.36% and 79.79%, significantly higher than the 58.79% and 53.29% of the unplanted control group, respectively. The results of Hussien *et al.* (2022) research's showed, total petroleum hydrocarbon (TPH) degradation percentage by *V. rosea* after a 5-month growth period ranged from 86.83 ± 0.44% to 59.05% ± 0.45% in soil treated with 1 and 7%, respectively. Plants raised in polluted soils demonstrated a dramatic reduction in germination rates, in addition to growth inhibition outcomes shown from decreased plant height. An increase in branching was observed with an increase in oil pollution percentages. Moreover, the phytomass allocated to the leaves was higher, while the phytomass witnessed lower values for fine roots, flowering and fruiting when compared to the controls. Panwar and Mathu (2023) investigated the potential of three phytoremediator plants species viz *Tagetes erecta*, *Helianthus annuus*, and *Medicago sativa* for remediation and translocation of individual PAH. The result showed that *M. sativa* significantly enhances the removal rate of PAHs in the soil. The dissipation rate reached 96.2% in *M. sativa* planted soil, followed by *H. annuus* and *T. erecta*. Among the plant species, *M. sativa* exhibited the highest root and shoot concentrations (314.37 and 169.55 mg kg^{-1}), while the lowest concentration was 187.56 and 76.60 mg kg^{-1} in *T. erecta*.

Because of daily increasing petroleum sludge in Isfahan oil refinery of Iran and also the necessity of refining contaminated soils, this research is carried out (1) To investigate the growth response of ornamental tall fescue and sunflower to contaminated soils with petroleum sludge and (2) To evaluate the phytoremediation potential of these two plant species to select a plant species compatible with the environmental conditions of Isfahan Refinery, for use in the phase Industry.

MATERIALS AND METHODS

In order to investigate the ability of plant of ornamental tall fescue and sunflower to refinement contaminated soils, an experiment were conducted in split-plots arranged based on completely randomized design. In this experiment the main factor was petroleum sludge level (0, 10 and 20 % (w/w) and sub main factor was plant species (tall fescue and sunflower and no-plant as control). All experiments carried out in three replicates using 27 pots (3 kg weight) in greenhouse condition. Petroleum sludge has collected from sludge reservoir of Isfahan oil refinery and also not contaminated soil (control) from grounds around of the refinery by the random method from depth of 0 to 40 cm soil. The samples packed in glass containers in the vicinity of ice pieces and were transported to the laboratory. After drying in air, the soil samples were sieved by a 2 mm sieve and on all the samples with three replicates, tests was done to measure the texture (hydrometric method), pH, electrical conductivity, lime, organic carbon, total nitrogen, potassium, absorbable phosphorus, percentage of moisture and porosity soil according to the standard methods (Page *et al*., 1982; Bremner and Mulvaney, 1982) (Table 1).

Sludge samples were air-dried and mixed by the weight ratio of 0 (control), 10 and 20% (w/w) with uncontaminated soil. In order to uniformity, the mixture was irrigated up to field capacity two weeks and then the pots filled by mentioned proportions. Seeds were cultivated in the depth of 1cm of soil. Seed germination measured two weeks after cultivation. Irrigation was done according to the daily observation of the plants and in such a way that water does not leave the bottom of the pot. After 120 days of planting, soil samples were taken from rhizosphere soil to determine the total amount of petroleum hydrocarbons in plant treatments. Shoot heigh of plants was measured with a ruler. Then the studied plants were harvested, and the roots and aerial stems of plants were separated from each other and dried in an oven at 75 ºC for 48 hours, and was measured the traits of root and shoot dry yields of the plants.

Measuring the amount of total petroleum hydrocarbons in the soil

In order to determine the total concentration of petroleum hydrocarbons and some poly aromatic hydrocarbons in the soils, first extraction was done by soxhlet method with an equal ratio of n- hexane and dichloromethane (Christopher *et al*., 1988). The compounds separated in the previous step were dried and concentrated inside the rotary by vacuum for separate the solvent and concentrate the samples, then they were purified by column chromatography using a silica gel and alumina column. The total concentration of petroleum hydrocarbons in the soil and petroleum sluge was determined by using the GC-FDI device and according to the 831 method of the US Environmental Protection Agency (U.S. EPA, 1984) (Table 1).

Table1. Some characteristics of soil (control) and petroleum sludge.

Parameter	Soil (Control)	Petroleum Sludge
pH (1:2)	7.8	6.85
EC (dS/m)	2.1	14.1
Lime (%)	40	31
OC (%)	0.41	15.1
N (%)	0.03	2.3
P (mg/kg)	5.8	77
K (mg/kg)	120	179
Ni (mg/kg)	0.3	2.5
Cd (mg/kg)	0.1<	1.3
TPHs (mg/kg)	12<	153600

Determining the total number of soil and oil-eating bacteria

To determine the total number of bacteria in the soil, 1 g of soil was poured into a test tube containing 9 ml of sterile sodium chloride solution of 9 g/L. The mixture was shaken vigorously and dilutions of 10^{-1} to 10^{-8} were prepared. Then, the diluted solutions were transferred to the rich nutrient agar solid medium. The plates were placed in an incubator for 48 hours at a temperature of 25 ºC (Soleimani *et al*., 2010).

Statistical analysis of data

In this research, the statistical design were conducted in split-plots arranged based on completely randomized design. After collecting and recording the data in the Excel software, analysis of variance (ANOVA) of datas was performed using SPSS 18 software and the means compared by LSD fisher test in level of 1%.

RESULTS AND DISCUSSION

The effect of petroleum pollution on plants germination

The results of analysis of variance showed that the usage of petroleum sludge in soil statistically affected on the percentage of plant germination (Table 2) ($P<0.01$).

Table 2. Analysis of variance of the petroleum pollution effect on seed germination.

S.o.V	df	MS
Petroleum sludge level (PSL)	2	172.041**
Error A	6	5.999
Plant	1	3567.542**
Plant × PSL	2	48.722**
Error B	6	0.750

**: Significant at $P < 0.01$ based on the LSD test.

The results of means comparison showed that in sunflower the germination percentage in 10 and 20% treatments of petroleum sludge has no significant differences as compared with control ($P<0.01$), but in tall fescue, seed germination statistically differed in all treatments in comparison with control (Fig. 1). The germination percentage of sunflower in 10 and 20% of petroleum sludge is higher at same treatments in tall fescue (Fig. 1). The lowest germination percentage was observed in tall fescue (9.3%) at 20% level of petroleum sludge, but the highest germination percentage was related to the sunflower (75%) in control (Fig. 1). It seems that in tall fescue the existence of petroleum contamination caused delay in seed germination. Sunflower which are resistant to environmental stresses so petroleum sludge-induced stress had lower effect on germination percentage. Similarly, it is reported that existence of petroleum contamination in soil has no significant effects on germination of different species of sunflower (Panwar and Mathu, 2023; Adewole *et al*., 2010). Wang *et al*. (2022); Zheng *et al*. (2021); Geo *et al*. (2017) and Liu *et al*. (2010) in investigation of rygrass germination in contaminated soil to diesel hydrocarbon found that by increasing petroleum hydrocarbons concentration, seeds germination percentage of this plant decrease significantly. They claimed that existence of petroleum hydrocarbon in soil act as oil layer surrounded the seed and avoid water imbibitions so caused the decrease in seed germination or seed vitality. The results of these researchers are consistent with the results of the present research.

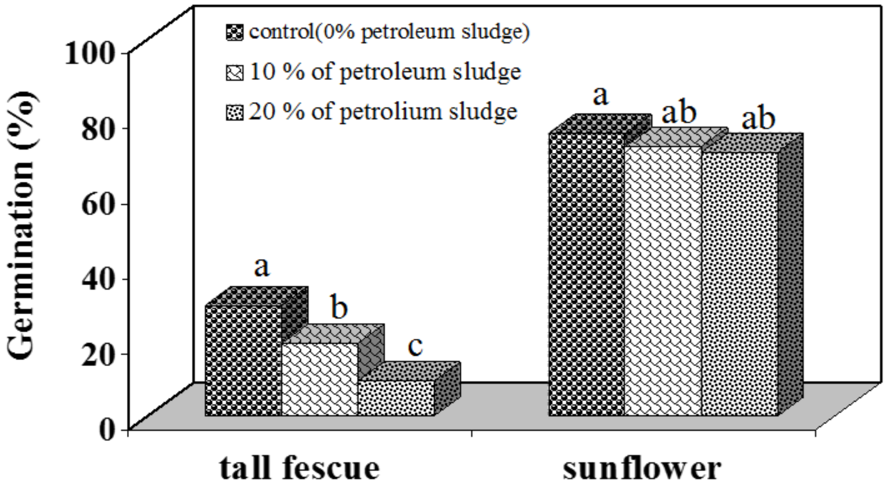

Fig. 1. Germination rate of seeds in different levels of petroleum sludge. (Means with similar letters are not significant in 1% probability level).

Changes in height of plants

The variance analysis of different percent of petroleum sludge on the height of plants is significant at statistical 1% level (Table 3).

Table 3. Analysis of variance of the petroleum pollution effect on height of plants.

S.o.V	df	MS
Petroleum sludge level (PSL)	2	7204.637**
Error A	6	10.772
Plant	1	4502.361**
Plant × PSL	2	396.717**
Error B	6	5.760

**: Significant at $P < 0.01$ based on the LSD test.

Results of means comparison (Fig. 2) showed that in tall fescue plan there is a significant reduction in the height of plant in treatment 20% of petroleum sludge at compared to control treatment ($P<0.01$) but in treatment 10% this reduction was not significant than to control treatment ($P>0.01$). In sunflower plant in every three levels of usage of petroleum sludge in soil there is a significant difference at 1% level on the reduction of height of this plant (Fig. 2), so that rate of decrease of height this plant in treatment 20% petroleum sludge is more than treatment 10% in comparison to control treatment. So, it seems that although sunflower plant has appropriate germination (Fig. 1) but existence of petroleum sludge in soil with different concentration reduce the growth and height of this plant and this reduction is more obvious for higher concentration of petroleum sludge. However, although tall fescue plant has low germination power in contaminated soils with petroleum sludge but existence of petroleum hydrocarbon has low effect on its growth. Nguemte *et al*. (2018); Kaimi *et al*. (2015); Adewole *et al*. (2010) and Ravanbakhsh *et al*. (2009) investigated the growth of grasses, sunflower and legume in contaminated soils to petroleum hydrocarbon compounds, their results show that although petroleum contamination influenced germination of grass species but this effect was not to the extent that avoid growth or disturb it severely. On the other hand, poison caused by petroleum hydrocarbon in soil has affect on growth (height of plants) of sunflower and legume and had significant reduction in its growth when concentration of petroleum compound has increased. The results of this research follow the results of the above reports.

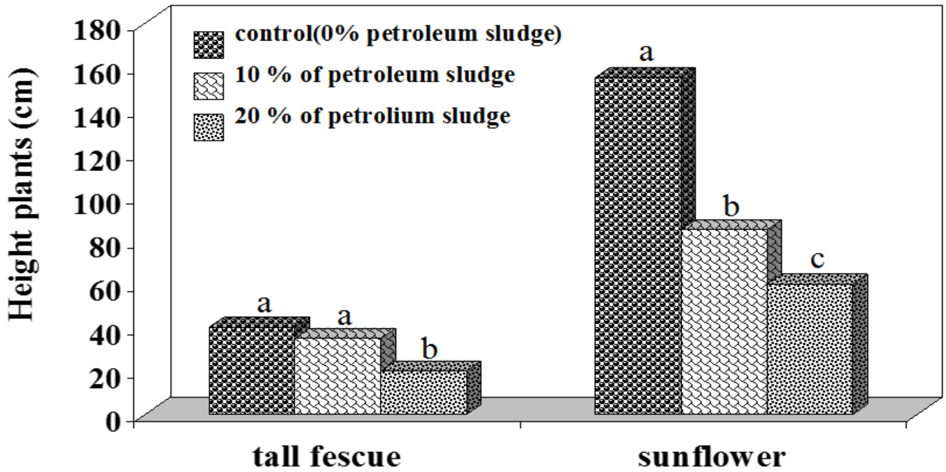

Fig. 2. Height of plants in different levels of petroleum sludge. (Means with similar letters are not significant in 1% probability level).

The effect of petroleum pollution on shoots and roots yield

The variance analysis of petroleum sludge effect on roots and shoots yield is briefed in Table (4). Results show that petroleum sludge application in soil has significant effect on root and shoot yield at 1% level.

Table 4. Analysis of variance of the petroleum pollution effect on shoots and roots yield.

S.o.V	df	MS (Root yield)	MS (Shoot yield)
Petroleum sludge level (PSL)	2	22.439**	78.931**
Error A	6	0.038	0.171
Plant	1	0.603**	6.035**
Plant × PSL	2	0.075**	0.912**
Error B	6	0.007	0.030

**: Significant at $P < 0.01$ based on the LSD test.

Results of means comparison showed that the highest yield of root (2.4 g/pot) and shoot (9.2 g/pot) observed in control treatment of tall fescue, although there is no significant difference between control and 10% treatment of petroleum sludge (Figs. 3 & 4). It seems that low amounts of petroleum sludge increased the yield of root and shoots by improvement physical condition such as increasing available water and improvement some condition of fertility and chemistry soil (Hussein *et al*., 2022; Adam and Duncan, 2002). By increasing petroleum sludge more than 10%, yield root (61.9%) and shoot (51.7%) in tall fescue decreased significantly at 1% level (Figs. 3 & 4). It seems that at the higher level of sludge (more than 10%) toxic compounds like polycyclic aromatic hydrocarbon caused significant reduction in root and shoot yields (Wang *et al*., 2022; Afegbua and Batty, 2018). So, petroleum sludge level of 10% can be chosen as the highest level of sludge which has no significant effect on tall fescue yield.

In sunflower plant, root and shoot yield mean had significant difference in both 10 and 20% levels of petroleum sludge in comparison with control at 1% confidence (Figs. 3 & 4). In other words, the existence of petroliuem sludge in the soil has reduced the mean of root and shoots dry weight of sunflower. The reduction of root and shoot yield at this plant in the 20% level of petroleum sludge was more than the 10% level (Figs. 3 & 4). As can be seen from the Figs 3 & 4, by increasing petroleum sludge from 0% level to 20%, level the dry weight of root and shoot of sunflower decreased by 83.6% and 75.7%, respectively. However, sunflower plant has higher germination rate as compared with tall fescue in these contaminated soils to petroleum sludge (Fig. 2), but it have lower resistance to petroleum contamination through growth period and it yield were trivial at the end of experiment (Figs. 3 & 4).

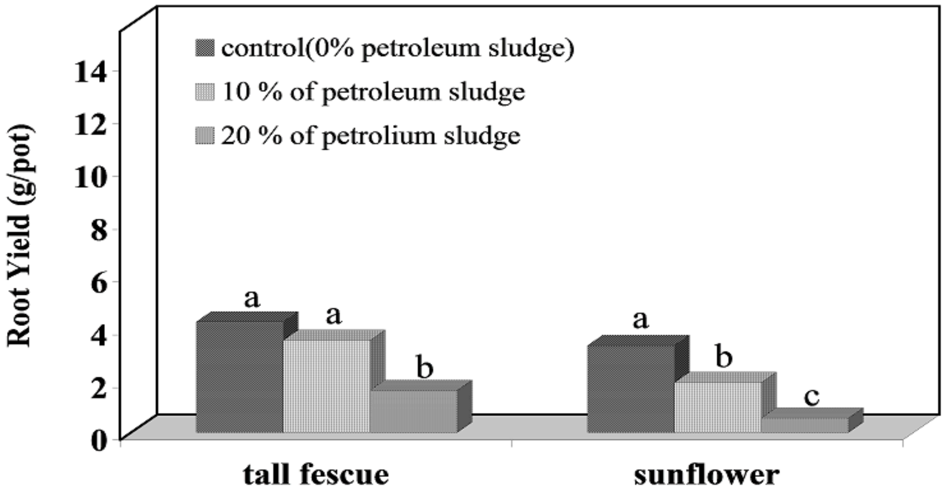

Fig. 3. Roots yield of plants in different levels of petroleum sludge. (Means with similar letters are not significant in 1% probability level).

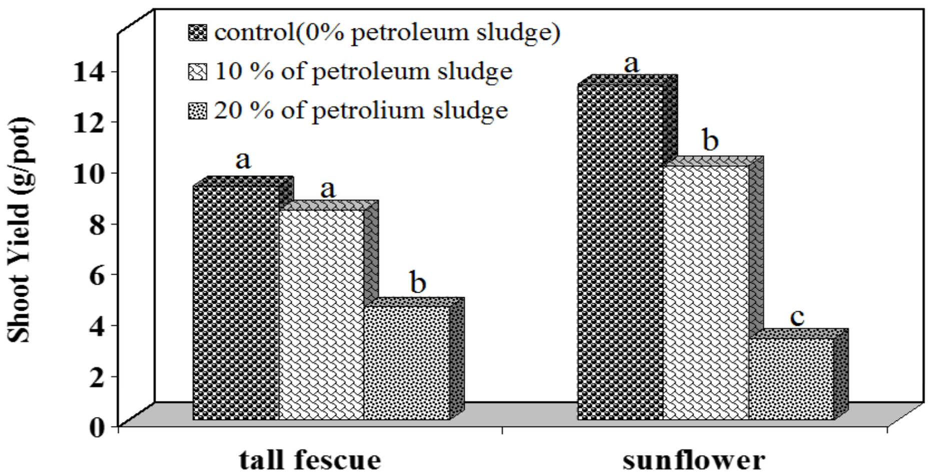

Fig. 4. Shoots yield of plants in different levels of petroleum sludge. (Means with similar letters are not significant in 1% probability level).

There is thought that soil toxicity by petroleum compounds and their effects on water absorption and nutrition limitation caused reduction of roots and shoots yield of sunflower plant (Panwar and Mathur, 2023; Xiao *et al*., 2015; Iraji Asiyaabadi *et al*., 2015; Qixing *et al*., 2011). So, using sunflower plant for phytoremediation of contaminated soils is not recommended in studied area. However, tall fescue has low germination ability in contaminated soils but petroleum hydrocarbon has lower toxic effect on its growth, root and shoots yields. So, because of its properties application of tall fescue is recommended for phytoremediation of contaminated soils of studied area.

Changes in total petroleum hydrocarbons (TPH)

The variance analysis of total petroleum hydrocarbons reduction is briefed in table 5. Results showed that application of plants in soil statistically decreased percentage of total petroleum hydrocarbon (P<0.01).

Table 5. Analysis of variance of reduction total petroleum hydrocarbons.

S.o.V	df	MS
Petroleum sludge level (PSL)	1	5164.818**
Error A	4	0.924
Plant	2	4625.597**
Plant × PSL	2	271.195**
Error B	8	0.629

**: Significant at P < 0.01 based on the LSD test.

The means comparison showed (Fig. 5) that there were statistically differences between sunflower and tall fescue in reduction of total petroleum hydrocarbon in both levels of petroleum sludge (P<0.01). Also, the reduction of total petroleum hydrocarbons in plant cultivated soils for each of petroleum sludge levels were higher than plant free soil which given as control (Fig. 5), so that on average about 21.5% reduction in total petroleum hydrocarbons was observed in the control treatment (Fig. 5). Many studies showed that decomposition of hydrocarbon contaminations in presence of plant is faster than no plant condition (Wang *et al*., 2022; Ali Khan *et al*., 2021; Afegbua *et al*., 2018; Kaimi *et al*., 2015). By regarding this fact that decomposition ability is differed between plants, according to this research TPH reduction in tall fescue is significantly higher in both levels of petroleum sludge than sunflower (P<0.01) (Fig. 5). The highest reduction of TPH (89%) is observed in tall fescue at petroleum sludge of 10% level (Fig. 5). This is because of higher root to shoot ratio in tall fescue than sunflower. Yuanyuan *et al*. (2020) and Merkel *et al*. (2005) in investigation of a mixture of *Festuca* L. and raygrass role in TPH reduction in soils contaminated to petroleum hydrocarbons found that the increase in root to shoot ratio caused higher TPH decomposition rate by aeration, root exudation and soil water balance. Geo *et al*. (2017) and Qixing *et al*. (2011) reported that fibrous roots provide more appropriate environment with higher specific surface for microbial activity. For this reason tall fescue plant by its fibrous roots redounded to induction of microbial activity and higher decomposition of petroleum contaminations. Similar to our study, Zheng *et al*. (2021); Nguemte *et al*. (2018) and Geo *et al*. (2017) reported that raygrass, bermodagrass and wheatgrass roots caused increase in hydrocarbon decomposition and reduction of soil toxicity. By increasing petroleum sludge more than 10% level, hydrocarbon decomposition significantly at 1% level decreased in applicated plants rhizosphere (Fig. 5). Its seem that increase in soil petroleum sludge level more than 10% create high toxicity for plants and microorganisms and reduced hydrocarbon decomposition (Zeng *et al*., 2021; Sarma *et al*., 2019; Adam and Duncan, 2002).

Sunflower plant showed the lower effects on hydrocarbon decomposition in both sludge level. So that can be seen from Fig. 5, decomposition amount of TPH in 10 and 20% petroleum sludge level were 55 and 30% respectively, that was because of lower biomass production, root exudates and microbial activity in this plant than tall fescue (Tejeda-Agredanoa *et al*., 2013). Panwar and Mathu (2023) and Hussein and *et al*. (2022) in investigating on petroleum sludge remediation reported that sunflower (*Helianthus annuus*) and *Vinca rosea* L. had low effects on TPH reduction in soils. They reported 30 to 53% reduction of TPH in presence these plants in comparison with no-plant condition. Also, similar effect was reported by Rogers *et al*. (2008) in four legume. The research results of these researchers are consistent with the results of the present research.

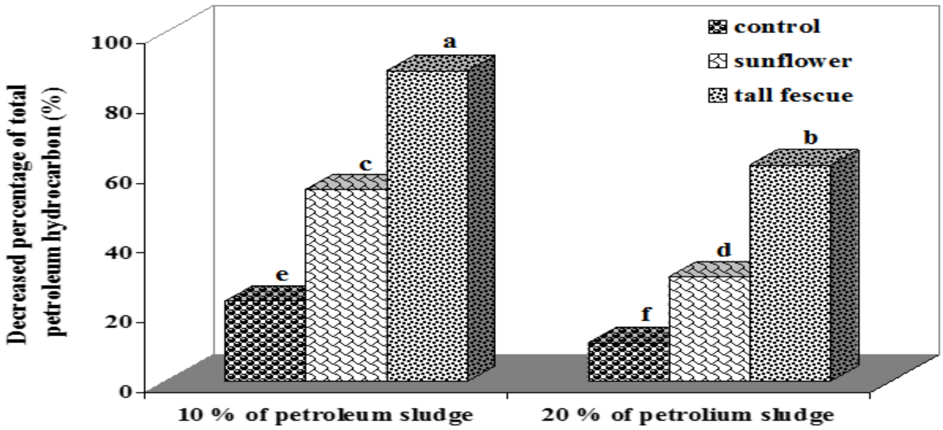

Fig. 5. Decreased percentage of total petroleum hydrocarbon in different levels of petroleum sludge. (Means with similar letters are not significant in 1% probability level).

The effect of petroleum pollution on the population of decomposing bacteria

The results of analysis of variance of the main effects of petroleum sludge application, plants and their interaction effects had a significant effect on the population of petroleum sludge decomposing bacteria at 1% level (Table 6).

Table 6. Analysis of variance of population of petroleum sludge decomposing bacteria.

S.o.V	df	MS
Petroleum sludge level (PSL)	1	1474.560**
Error a	4	1.691
Plant	2	105.377**
Plant × PSL	2	31.739**
Error b	8	0.769

**: Significant at P < 0.01 based on the LSD test.

Fig. 6 showed the results of means comparison effect of the use of tall fescue and sunflower on the population of petroleum sludge decomposing bacteria at the end of the growing season for the application of different levels of petroleum sludge. Can be seen that the lowest number of petroleum sludge decomposing bacteria related to the condition without plant cultivation that in first was observed at 20% level of sludge (2.8×10^6 CFU/g soil) and then at 10% level of sludge sludge (1.4×10^6 CFU/g soil). The results of many researches such as the studies of (Wang *et al*., 2022; Ali Khan *et al*., 2021; Afegbua *et al*., 2018; Kaimi *et al*., 2015) showed that the number of petroleum sludge decomposing bacteria is higher in the presence of plants than no plant condition. Of course, the number of petroleum sludge decomposing bacteria in the presence of different plants is variese, so that according to results the present research at the level usage of 10% sludge the highest number of petroleum sludge decomposing bacteria is related to cultivation of tall fescue plant (16.9 × 10^6 CFU/g soil) and then related to sunflower cultivation (9 × 10^6 CFU/g soil) and in this condition, the difference between these two plant was statistically significant at the 1% level (Fig. 6). The increase in the number of decomposing bacteria is probably due to the role of pollution as a substrate for the microbial community and the high activity of native microorganisms of soil. Also, the presence of plants through the release of nutrients and their secretions in the soil and the transfer of oxygen to the root area has stimulated and increased the activity of the microbial population that decomposes petroleum pollutants (Zeng *et al*., 2021; Geo *et al*., 2017; Iraji-Asiyaabadi *et al*., 2015).

By increasing the amount of sludge consumed to more than 10%, the number of bacteria that decompose petroleum hydrocarbons in the rhizosphere of tall fescue (30%) and sunflower (50%) showed a significant decrease at 1% level (Fig. 6). It seems that the excessive increase of sludge in the soil (more than 10%) causes a lot of toxicity for microorganisms and reduces the number of bacteria that decompose hydrocarbons sludge (Zeng *et al*., 2021; Sarma *et al*., 2019; Adam and Duncan, 2002). A comparison of the percentage of reduction of total petroleum hydrocarbons for the studied plants (Fig. 4) with the number of petroleum sludge decomposing bacteria (Fig. 5) showed that the trend of changes of these two parameter in both levels of applied sludge in the soil are similar with each other. Studies Nguemte *et al*., 2018; Iraji-Asiyaabadi *et al*., 2015 and Liu *et al*., 2010 showed that the decomposition rate of petroleum hydrocarbons in the soil is related to the number of decomposing bacteria. Also, the studies Chen *et al*., 2023; Zhang *et al*., 2022; Jiang *et al*., 2022 and Tejeda-Agredanoa *et al*., 2013 showed that the total amount of petroleum hydrocarbons remaining in the soil has a negative correlation with the number of soil microorganisms, and the most reduction of petroleum hydrocarbons is in the soil rhizosphere, which is due to the increase in the number of soil microorganisms in this area. The results of these researchers are matching with the results of the present research.

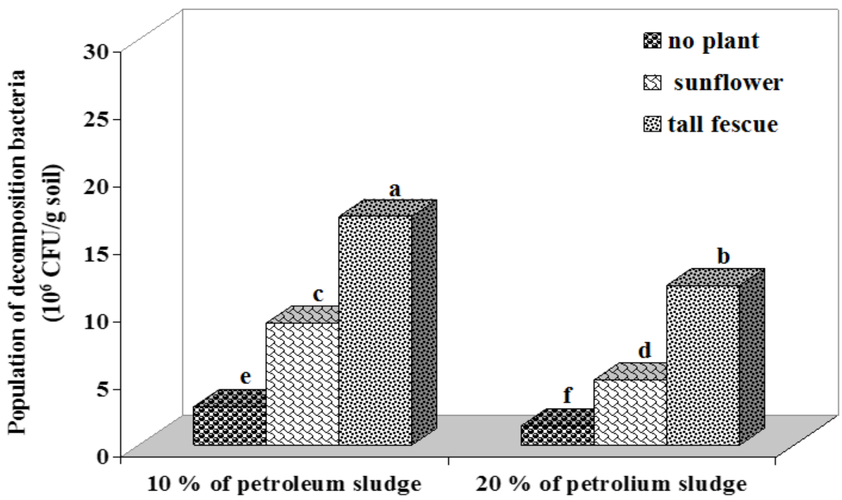

Fig. 6. Population of decomposing bacteria in different levels of petroleum sludge. (Means with similar letters are not significant in 1% probability level).

CONCLUSION

The results of current investigation on germination rate, growth, root and shoot yield of sunflower and tall fescue showed that germination rate of sunflower was higher than tall fescue, but root and shoot yields of this plant were lower significantly than tall fescue in both petroleum sludge levels. So, in selecting plants for phytoremediation only relying on germination of plants is not good indicator for plants establishment and determination of growth ability, survival and adaption of plant to petroleum contamination is very essential. In presence of studied plants and different petroleum sludge levels, TPH amounts reduced in comparison with control (no-plant). The highest reduction (89%) is observed in 10% level petroleum sludge in tall fescue rhizosphere. By increasing the amount of petroleum sludge to more than 10% level, hydrocarbon decomposition reduced significantly in the studied plants rhizosphere. So, according to high growth, yield and root to shoot ratio in tall fescue which caused more reduction of total petroleum hydrocarbons in soil, this plant is recommended to phytoremediation of petroleum sludge at the level of 10% petroleum sludge of usage in soil studied region.

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