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THE EFFICIENCY OF BARLEY *HORDEUM VULGARE* IN PHYTOREMEDIATION OF CRUDE OIL - CONTAMINATED SOIL

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ABSTRACT

This study aimed to evaluate the phytoremediation potentiality of barley *Hordeum vulgare* L. that could be used to remediate hydrocarbon from the contaminated soils. A pot culture experiment was conducted taking 2 kg of field soil mixed with crude oil at a concentration of 25, 50, 75, and 100g.kg^{-1}. Ten healthy seeds of barley were sown in pots of each concentration for germination. A control setup was maintained without adding crude oil. The duration of the experiment was fixed for three months. The results showed that the plants' uptake of hydrocarbon increased with increasing crude oil concentration in the soil. The results also demonstrated a reduction in plant shoot and root biomass with an increase in crude oil concentration. Furthermore, the results revealed that the shoot biomass was higher than root biomass in all the treatments. BCF of barley was the highest in low concentration treatment.

KEY WORD : Barely, Petroleum hydrocarbons, Phytoremediation, Soil quality.

INTRODUCTION

Petroleum hydrocarbons PHCs pollution is one of the most important environmental problem. Petroleum and its derivatives are the mixtures of gaseous, liquid, and solid hydrocarbons. The elementary naturally occurring reservoirs of crude oil are thus homonym as petroleum hydrocarbons PHCs. PHCs can be broadly divided into two families: aliphatic fatty and aromatics fragrant Chukwuma *et al*., 2012. Petroleum hydrocarbons establish in the environment from natural and human activities, but their contamination is usually associated with anthropogenic activities Dreyer *et al.,* 2005; Okere and Semple, 2012; Sayara *et al*., 2011. PHCs oblige a significant ecological risk because they have tolerated toxic, carcinogenic, mutagenic, teratogenic effects and are resistant to biodegradation (Sayara *et al.,* 2011).

Contamination of existing agricultural fields is a significant problem associated with the processing and distribution of crude and refined petroleum hydrocarbons in many oil-producing countries. This has led to exploring many remedial approaches to affect the clean up of polluted agricultural soils and restoring them to near original conditions. Biodegradation of these wastes is becoming an increasingly important treatment method. Its advantages include low-cost equipment, environmentally friendly nature of the process, and simplicity Azubuike *et al*., (2016). This has prompted research into factors that could create the best conditions for accelerated biodegradation.

Phytoremediation is an environmentally friendly use of vegetation to remove pollutants (Lee, 2013; Azeez, 2021). Plant-based technology can aim in both inorganic and organic pollutants, and in recent years, a considerable interest in surveying the phytoremediation of co-contaminated soils emerge (Ouvrard *et al.,* 2011; Chigbo *et al*., 2013; Sung *et al*., 2013). Phytoremediation is an attractive hopeful method for treating PHCs contaminated soils because of its cost-effectiveness and ecological advantages (Bell *et al.,* 2014; Kukla, P³ociniczak, and Piotrowska-Seget, 2014).

Tang and Angela, (2019) study contributes to identifying four local, readily-available plant species and their effectiveness for cost-effective phytoremediation of crude oil-contaminated soil. Phytoremediation of polluted soil with Iraqi crude oil using grass plant was studied by Al-Obaidy *et al.*, (2018). There are a few studies on this subject in Iraq.

Considering previous studies on oil contamination and the target soil properties, we selected *Hordeum vulgare* for phytoremediation of oil-contaminated soils around Basrah oil fields.

MATERIALS AND METHODS

Experimental design

The soil was collected from the Basra agricultural fields, divided into six treatment pots in a greenhouse. The beds were necessary to prevent excessive run-off of the crude oil to nearby pots. The pots were spaced one meter apart to check the effect of side seepage of the contaminants and applied water from one pot to another. Pot O was the control without any treatment. Pots A, B, C, D, and E were labeled for the addition of 2 kg of silty clay into each experimental pot, using crude oil concentrations in the experiment as follows 25g.kg^{-1}, 50g.kg^{-1}, 75g.kg^{-1}, and 100g.kg^{-1}. The PHCs contaminants were mixed by hand with the clean soils at specific ratios to obtain the designed gradient concentrations. The barley species *Hordeum vulgare* was selected for the phytoremediation study after screening-out and confirmatory experiments. The plants were harvested in each 30-day culture After 30 days T1, after 60 days T2, and after 90 days T3. Different random spots were augered with a hand soil auger, bulked together for composite samples, and put in labeled polyethylene bags. This procedure was done three times to form three replicates for chemical determination. The samples were immediately transferred to the laboratory for analysis.

Soil physical and chemical properties

The soil samples from the contaminated and clean soil were prepared for analysis. It was air-dried at room temperature, homogenized with a pestle and mortar, and sieved through a 1.00-mm mesh

Assessment of soil pH, EC, and moisture

Soil pH and electrical conductivity EC of soil were determined by using a Hana meter in 1:1 soil\water. Soil moisture was determined as ten grams was taken from a soil sample relative to the dry soil weight. The moisture content in the soil was measured in percentage Estefan *et al*., (2013).

Assessment of total organic carbon

The total organic carbon content was measured by the incineration method. Estimation of total nitrogen was done depending on Sumner *et al.*, (1996) for evaluation of the organic matter as:

Organic matter % = 1.724* Total organic carbons%

C: N Ratio in soil

The carbon to Nitrogen ratio was calculated as described by Estefan *et al.*, 2013:

C\N ratio = Organic carbons\Total nitrogen……

Determination of plant Properties

Root and shoot lengths were measured by removing and separating the plant parts from the soil very carefully. After that, plant parts were washed and blotted to remove the excess amount of water. After collecting the plant directly, wet weight was estimated, then dry weight by drying plant samples in an oven at 75 °C for 72 hours.

Total chlorophyll

Determination of total chlorophyll of the plant leaves based on Arnon (1949) was done by taking one gram of fresh leaves and ground with 40 ml of acetone 80%. It was centrifuged at 5000 rpm for five minutes. The supernatant was transferred, and the procedure was repeated. The absorbance A was read at 645nm and 663 nm against a blank. The total chlorophyll was calculated using the equation

total chlorophyll= $20.2A_{645} + 8.02A_{663}$ ……

Estimation of Total PHCs

Soxhlet estimated the total petroleum hydrocarbon PHCs content of the soil and plant samples both in roots and shoots. Estimation TPH was done depended on Goutx and Saliot 1980 and Grimalt and Oliver, 1993.

Statistical analysis

The experiment was conducted using arandomized complete block design with threereplications. SPSS v.20 was used as a statistical program for data analysis. Variances were homogeneous according to Bartlett's test P = 0.05. Pearson correlation coefficients were calculated.

RESULTS

Table 1 showed the characteristic of soil – control treatment. The tested plant in the current phytoremediation study showed good behavior in petroleum hydrocarbon-contaminated soil. Table 2 showed soil from polluted pots with a concentration of 25 $g.kg^{-1}$, 50$g.kg^{-1}$, 75$g.kg^{-1}$, and 100 $g.kg^{-1}$.

Table 1. Physical and chemical characteristics of the soil used as control.

Measurement	Unit	Value
pH		7.2
Electrical conductivity EC	mS/cm	2.4
Moisture	%	4
TOC	%	2.15
Total N	%	2
Organic matter	%	3.7
C:N		1.07
PHCs	µg/g	0

Seeds germination

The percentage of seeds germination was measured by examining the seedling sprouting after nine days of seeds sown in treatment pots compared with that of the control. The results showed that crude oil took adverse effects on barley seed germination. With the oil concentration increasing, the germination rate of barley seed decreased respectively Figure 1. The correlation coefficient was r=0.9 P<0.05

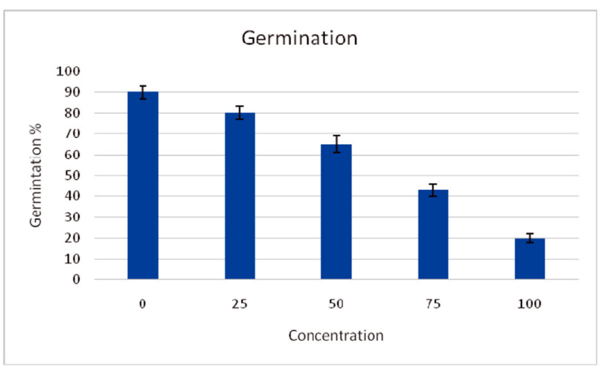

Fig. 1. Percentage of seeds germination % during the study period.

Root and shoot lengths

The root and shoot lengths were decreased in plants exposed to crude oil concentrations compared to the control treatment; the plant grown in soil contaminated with crude oil showed sensitivity to crude oil with a reduction in its root and shoot lengths. The highest inhibition in root and shoot lengths was evident in 75$g.kg^{-1}$ and 100g\kg concentrations, respectively Figure 2. A significant negative relationship was found r=-0.65 p <0.05.

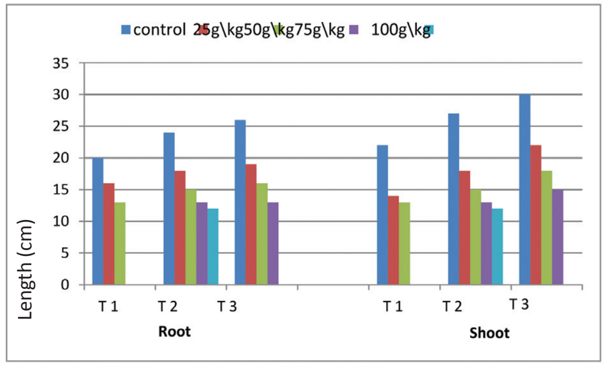

Fig. 2. Root and stem length for the plant with different concentration of crude oil

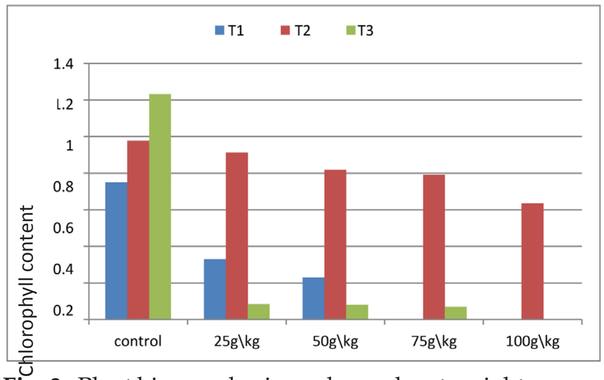

Fig. 3. Plant biomass basis on dry and wet weight among different treatments

Total chlorophyll

The plant is grown in oil-polluted soil generally showed chlorophyll deficiency in leaves; it decreases with increasedconcentration and refers to plant health. *P<0.05*. Figure 4.

The plant biomass

Fresh and dry weight decreased significantly with an increase in the level of contamination figure 3. Plant biomass in contaminated pots was less than in

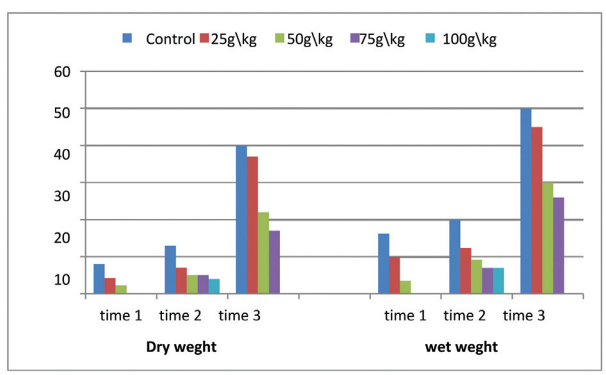

Fig. 4. Total chlorophyll of tested plant during different times

control pots. However, with increasing contamination level significant decline in fresh and dry weights occurred compared to the control. The experimental plant revealed that after harvest, the biomass was higher in control and lowered in the following concentrations. The reason may be that the crude oil concentration in soil decreased the growing plant and inhibition photosynthesis. Significant variation was recorded fresh weight $p<0.05$, dry weight $P<0.05$.

Accumulation of Total PHCs

The highest PHCs removal rate was observed within the final month T3 of the experiments in all tested pots Figure 5. The removal rate decreased with an increased concentration of crude oil. The efficiency of phytoremediation experiments was always

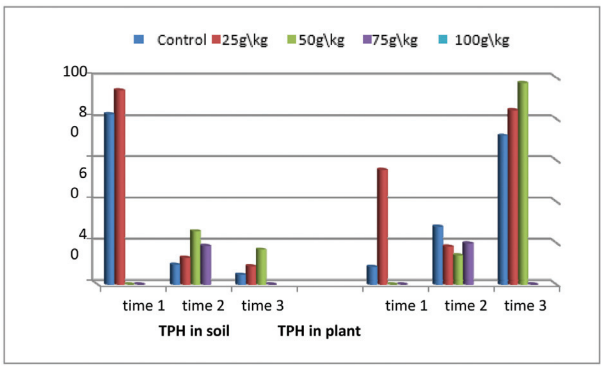

Fig. 5. Total PHCs in soil and plant during the study

statistically higher than the control.Significant effect of studied plants on petroleum hydrocarbon removal at different sampling times $P<0.05$.

Table 2. The removal rate of TPH within the different treatments

Concentration of PHCs	Removal %
25 g/kg	95%
50 g/kg	91%
75 g/kg	83%
100 g/kg	81%
No plant with 50g\kg	42.49%

Bioconcentration factor

The ability of plants to accumulate hydrocarbons in tissue as follows. Table 3 showed BCF of barley species in different concentrations.

Table 3. Bioconcentration factor of barley specis in different concentration of crude oil

BCF\CON.	25	50	75	100
BCF	16.38	9.77	5.99	1.06

DISCUSSION

This study showed that hydrocarbons have a common effect on the plants physiologically, physically besides the soil's physical and chemical

Table 2. Physical and chemical characteristics of the experimental soil used in the study during different treatments time.

Analysis	Treatment	control	25 $g.kg^{-1}$	50 $g.kg^{-1}$	75 $g.kg^{-1}$	100 $g.kg^{-1}$	P-value
pH	T1	7.4	7.2	7.2	7.1	7.1	P<0.05
	T2	6.5	6.5	6.4	6.4	6.4	
	T3	7.5	7.3	6.8	6.8	6.7	
EC	T1	2.1	2.3	2.9	2.9	3	P<0.05
	T2	1.22	1.56	1.65	1.87	2.9	
	T3	2	2.3	2.3	2.4	2.6	
Moisture	T1	4.97%	4.96%	4.95%	4.95%	5.0%	P<0.05
	T2	5%	5%	4.90%	4.90%	4.90%	
	T3	5.50%	5.30%	5.20%	5.20%	5.00%	
TOC	T1	2.1%	2.2%	2.5%	2.5%	2.6%	P<0.05
	T2	1.8%	1.9%	1.9.6%	2.8%	2.8%	
	T3	1.8%	1.9%	1.9%	2.0%	2.3%	
Total N	T1	1.80%	1.90%	2.10%	2.20%	2.30%	P<0.05
	T2	1.55%	1.64%	1.69%	2.20%	2.30%	
	T3	1.10%	1.30%	1.50%	1.50%	1.70%	
Organic matter	T1	3.6%	3.7%	4.3%	4.3%	4.4%	P<0.05
	T2	3.1%	3.27%	3.37%	4.8%	4.5%	
	T3	2.1%	2.6%	3.1%	3.2%	3.6%	
C: Nratio	T1	1.16	1.15	1.19	1.13	1.13	P<0.05
	T2	1.91	1.85	1.92	2.06	2.08	
	T3	1.2	1.18	1.18	1.20	1.27	

properties. It was found that the plant has a high capacity to remove hydrocarbons from the soil in the different concentrations. Low concentrations were removed more rapidly than in high concentrations hydrocarbons. This is agreed with the conclusion of the Saraeian, 2018.

Barely *Hordeum vulgare* has a high potential for hydrocarbons' bioaccumulation, depending on hydrocarbons' concentration. This agreed with Zhan 2014. It was found that the toxic effect of hydrocarbons on the plants affected the process of photosynthesis and reduced its production.

In contrast, the plants that grew in control were higher in biomass weight due to the absence of hydrocarbons. This is consistent with Cheema *et al.,* 2010 and Alwan, 2015.

The plant soil's pH in the control pot was higher than that in pots containing different hydrocarbons concentrations. This result agreed with Abbaspour *et al.,* 2008. Plant soil's electrical conductivity is less in control pots than the pots' containing different hydrocarbons concentrations. The barley plant soil's moisture content in pots containing different hydrocarbons concentrations was less than recorded in control soil. This resulted in an agreement with Macoustra *et al.,* 2015.

This study indicated that the barley seeds had a different germination and growth rate of seedlings. It decreased by increasing crude oil concentration, while germination in the control pot was the highest. These results are consistent with Sivaram, 2018. The barley plant's root length differed among the pots containing different hydrocarbons concentrations, where the length of the root in the control pot was found to be the longest. In contrast, the root showed the effects of hydrocarbons in the pot containing different concentrations of hydrocarbons. The root length is inversely proportional to the concentration of hydrocarbons. This study agreed with the findings of Visioli *et al.,* 2014.

The barley grows in the pots containing different hydrocarbons concentrations with a shorter shoot than plant growth in the control pot.

This study showed that hydrocarbon oil contains 85%-87% organic carbon. The TOC value in the high concentration of hydrocarbons was higher than the control pot. This result agreed with Macoustra *et al.,* 2015. The study showed that hydrocarbons' addition had caused the increase of nitrogen in the soil; this is consistent with the findings of Eifediyi and Remison 2010.

The current study showed a variation in the C/N ratio of barley soil between pots containing different concentrations of oil hydrocarbons and control pots. This is consistent with Basatumary, 2012.

CONCLUSION

It can be concluded that there was the efficacy of the plant used in phytoremediation. Plant ability in the process of accumulation of petroleum hydrocarbons with diverse effects.

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