

Seed germination and Phytoremediation of some plants in Contaminated Soil with Petroleum Hydrocarbon in Basrah City

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Abstract:

Fifteen plant species were collected from local markets of Basrah City to evaluation the ability to germination and Phytoremediation for the removal of hydrocarbon contaminated soil near South Refineries company in Basrah city .The pre-pot germination test were done in Petri dish in room temperature (25-30°C) for two week before cultivated on the soil polluted with hydrocarbon, some of seed showed higher than 95%. Germination seeds were count and recorded daily at the end of two week. Seed growth in soil polluted has been done directly on soil in pots fraught with soil polluted and put in outdoor. Germination in polluted soil without any modification did not give any result compared to control samples. The results showed that after treatment of contaminated soil with hydrocarbons, some treatments, such as dilution with clean soil, animal manure, compost and sawdust, showed different germination rates for different types of seeds within 30 days. A number of plants were selected for use in subsequent experiments. During the study period, pH, electrical conductivity, N.P.K concentration and total number of air bacteria were measured. The results of the experiment showed that there are eleven species of plants have given the results of germination in the soils treated in different treatments. But most species have the ability to germinate in more than the treatment is (*Helianthus anuus*, *Trigonella foenum*, *Medicago sativa*, Red sorghum and *Triticum aestivum*), and also gave the ability to reduce the proportion of hydrocarbons during the period Experiment where the trial period was completed for three months and recording the concentrations of TPH. Seed germination is the most effective step to plant permanence, which in turn affects the success of the plant treatment process. In this Search we study the suitable plants for cleanup of hydrocarbon contaminated soil by Phytoremediation.

Key words: Phytoremediation; Contamination; Hydrocarbon –polluted soil; plant

1- Introduction

Contaminations from industrial, re-search experiments, military, and agricultural activities either due to ignorance, lack of vision, carelessness, or high cost of waste disposal and treatment are increasingly affected on Land, surface waters, and ground water worldwide. Major strains of ecosystems are affected by accumulation of toxic contaminated (metals, radionuclide, and organic contaminants in soil, body water, and ground water). Soil contamination with petroleum hydro carbon enable repair by variation process such as physio-chemical & biological methods (Tang et al., 2010). Bioremediation is thought to be least cost, least environmental hazard but longer time needed contrast to other process. Consequently, there is arising attended to evolution of Phytoremediation, which is friendly to environment, inexpensive & not need to energy. (Etsuko, et al., 2007). Phytoremediation is baste technology for remove contaminated from environmental

contamination has been growing rapidly in recent years. This process including use of green plant “tolerant plants” to remove toxic organic compound from soil and ground water. Phytoremediation include cultivation plants in a contaminated environmental to clean it by promote reservation and/or degradation (detoxification) of the pollutants (Lasat, 2000). However, phytoremediation has seen that various pollutants of oil products and solvent, are break down rapid in the existence of plants because the root zone are thought to be influencing factor which filtering of water , oxygen transport & biological stimulation in this reign (Tang et al., 2010)

Phytoremediation of contaminated soil is usually have one or more of the following techniques: Phytoextraction , Phytostabilization , Phytodegradation , Phytovolatilization , Rhizofiltration and Rhizodegradation (Table 1) (Wang et al., 2003 and Oh et al., 2013).

Table 1: Phytoremediation processes for remediation of contaminated soils.

Phytoremediation	processes Description
Phytoextraction	Plants absorb contaminants and store in above-ground shoots and the harvestable parts of roots
Phytostabilization	Roots and their exudates immobilize contaminants through adsorption, accumulation, precipitation within the root zone, and thus prevent the spreading of Plant contaminants.
Phytodegradation	Enzymatic breakdown of organic contaminants, both internally and through secreted enzymes.
Rhizodegradation (phytostimulation)	Plant roots stimulate soil microbial communities in plant root zones to break down contaminants.
Phytovolatilization	Contaminants taken up by the roots through the plants to the leaves and are volatilized through stomata where gas exchange occurs.

Therefore, many studies have been made to use of plants in phytoremediation technology to degraded organic contaminates such as polyromantic hydrocarbons (PAHs) (Aprill & Sims 1990 and Siciliano et al., 2003), polychlorinated biphenyls (PCBs) (Donnelly et al., 1994) and hydrocarbons (Banks et al., 2003a and Kirk et al., 2005) by using various plant species in North America and Europe. Sorghum, Italian regress, *Zea mays* and alfalfa are known as phytoremediators (Parrish et al. 2004 and

Banks et al., 2003b). Bermuda grass, sunflower, southern crabgrass & red clover are demonstration as hydrocarbon tolerant plant (Olson and Fletcher 2000). These research indicated that grass pieces and Leguminosae could be appropriate for phytoremediation of soil contaminated with petroleum hydrocarbons. Because fibrous root systems that have grass along roots that have a large surface area per unit volume of soil. Fibrous roots supply a great surface than taproots (Anderson et al., 1993). Nitrogen –

fixing bacteria have a symbiotic connection with Leguminosae, these connected indicated that Leguminosae can be germinated fully in soil contaminated with petroleum.

Etsuko et al. (2007) use twelve plants for screened of their phytoremediation ability for breakdown of hydrocarbons contaminated soil in Japanese, the research of screening that eight plant species gave very marked decrease in the TPH concentration. In Nigeria attempts to use plant to remediate oil polluted (Njoku et al., 2009; Efe & Okpal 2012). Whereas (Ayotamuuno et al., 2006) permeate in his study in Port Harcourt for six weeks, and confirmation through this work that *Zea mays* (Corn) and soil contaminated and establish the rate hydrocarbon break of 77.5% *Z. mays* and 83% during first two weeks.

Any wise, In order to use phytoremediation, it is important to choose plant species that are appropriated for

environmental conditions of site study. In this study, we choose fifteen plant species that can germinated in petroleum hydrocarbon contaminated soil and selected some of plant as phytoremediators.

2- Materials and Methods

2.1-Samples collection and soil properties

The petroleum hydrocarbon-contamination soil were collected from Oil South Refineries Company in Basra –Iraq. Sample was take from depth about 30cm, from four different site (randomly) and placed in clean plastic (pages) container. The soil was then blended well and saved with a 4-5 sieve to remove stones and debris in order to obtain uniform blend.

2.2-Plant species

Fifteen plants species used in this study, table (2) illustrate plant species analyzed.

Table (2) Plant species that used in Phytoremediation

No.	Genus and species	Common name	Family
1	<i>Medicago sativa</i>	Alfalfa	Legumnaceae
2	<i>Zea mays</i>	Corn	Graminaceae
3	<i>Helianthus annuus</i>	Sun flower	Compositaceae
4	<i>Trigonella fonom</i>	Grecum	Fabaceae
5	<i>Rhaphanus raphanistrum</i>	Radish	Brassicaceae
6	<i>Phaseolus vulagris</i>	Bean	Legumnaceae
7	<i>Linum</i>	Flax	Linaceae
8	<i>Triticum aestivum</i>	Wheat	Graminaceae
9	<i>Hordeum vulgare</i>	Barley	Graminaceae
10	<i>Sorghum bicolor</i>	Sorghum	Poaceae
11	<i>Dancus carota</i>	Carrot	Apiaceae
12	<i>Red sorghum</i>	Sorghum	Poaceae
13	<i>Vicia faba</i>	Faba bean	Fabaceae
14	<i>Vigna rosea</i>	--	Fabaceae
15	<i>Ricinum communis</i>	Caster bean	Euphorbiaceae

2.3-Pre-pot germination test in Petri dish

Pre –pot germination were done in prudish at room temperature (25-30°C) for two week before cultivated on the soil polluted with hydrocarbon, to evaluate the vitality of seeds plant.

2.4-Germination in polluted soil

Seeds growth in soil polluted has been done directly on soil in pots fraught with soil polluted and put in out door for two week and recorded results daily. The soil was weighed

and transferred to pots (300g of soil) and have size (8.5 cm length ×9.5 cm breath)

2.5-Germination in treated polluted soils

Before cultivated seeds in soil polluted with hydrocarbons some treatment have been done

such as dilution with clean soil, animal fertilizer, compost, saw dust that showed different germination rates for different types of seeds within 30 days, constituent of soil in pots was as follows in (Table 3)

Table (3) Types of Treated Soils and their cods

Treated soils	Cods
100% contaminated soil +Animal 5%fertilizer +5% saw dust	C1
100% contaminated soil +5% saw dust	C2
80% contaminated soil +20% clean soil	A
80% contaminated soil +20% animal fertilizer	B
50% contaminated soil +50% clean soil	E1
50% contaminated soil +50% clean soil +5%composte +5% Animal fertilizer	E2
80% contaminated soil +10% clean soil +10% animal fertilizer	F
80% contaminated soil +10% compost +10% saw dust	D

The soil was weighed and transferred to pots that the same used in (section 2.4) .The primary amount of TPHs in soil was detected in laboratory by gravimetrically method. There put 10 seeds cultivate in each pot.

2.6-Bacteria count

Serial dilution method was used to decide the numbers of bacteria in soil polluted. 1.0 g of soil transported to 100ml sterile water and mixed well on hot plate stirrer, serial dilution were designed up to 10^{-7} .then take 0.1ml from

10^{-4} , 10^{-5} , 10^{-7} and spread on surface plate contain N.A for bacteria, and incubated at (30-32°C) for (24- 48) hr.

2.7-Measurement of TPHs

T.P.Hs elicited by take 5g of dried soil was successively (serially) eluted with 15ml of hexane, Methylene chloride and chloroform. Solvent evaporation by pooled and dried at room temperature under gentle nitrogen stream in a fume hood. Gravimetrically method was used to evaluation amount of residual TPHs after solvent evaporation (Mishra et al., 2001)

3- Result and Discussion

3.1- Soil properties

Texture of soil is believed to have deep impact on a large characterizes was considered one of physical character as illustrate in table (4). On other hand pH is considered from important factor that effects on soil chemical and biological reactions are dominance by pH of soil. A major problem in the realization of

suitable plants. A crops of many plants are very effects when the pH rates are low(< 5.0) (NPSP, 2005). Electric conductivity may be impacted on seed germinated; however some plant not clear effected unto EC greater than 4 (dS) m^{-1} (McCutcheon and Schoor, 2003). EC of soil that used in this study had EC of (38.5 mv).

3.2- Pre- pot germination in Petri dish

The Percentage of germination in Petri dish was detected after 3,7 and 14 days in Petri dish temperature was about 25-30°C date experiment done in October 2018 this results show in Table (5) illustrated seeds germination for all plants .

The highest percentage germination was recorded for six seeds plants (*Medicago sativa*, *linum*, *Triticum aestivum*, *Hordeum vulgare* and *Rhaphanus raphanistrum*) during 3days , Whereas some seeds germinated after 7days with presently 100% (*Trigonella fonum* , *Vigna*, *Sorghum* and *bicolor Red bicolor*).

Table (4): Physical and chemical feature of experimental soil

Para meter	Value	Analytical method
pH	8.4	1:1 soil/water (Andrew <i>et al.</i> , 2005)
Moisture %	9.9	(Andrew <i>et al.</i> , 2005)
EC (mv)	38.5	1:2 soil/water(Andrew <i>et al.</i> , 2005)
Texture soil		
Sand (%)	10	(Folk,1974)
Silt (%) (sandy loamy)	77	
Clay (%)	13	
TPHs g/kg	36.3	(Mishra et al 2001)
Organic matter (%)	1.755	Standard method 2005
Organic Carbon	1.28115	Standard method 2005
Total N (%)	3.85	Standard method 2005
P(mg kg ⁻¹)	6.8	Standard method 2005
K(mg/g)	0.72	Standard method 2005

Table (5) Seeds Germination after 3, 7 and14 days in Petri dish

N o.	Seeds Plant	Germination%		
		3days	7days	14days
1	<i>Medicago sativa</i>	90	100	100
2	<i>Zea mays</i>	0	10	20
3	<i>Helianthus annuus</i>	0	0	0
4	<i>Trigonella fonum</i>	50	100	100
5	<i>Rhaphanus raphanistrum</i>	100	100	100
6	<i>Phaseolus vulagris</i>	20	20	20
7	<i>Linum</i>	100	100	100
8	<i>Triticum aestivum</i>	90	90	100
9	<i>Hordeum vulgare</i>	90	100	100
10	<i>Sorghum bicolar</i>	50	80	100
11	<i>Dancus carota</i>	20	90	100
12	<i>Red bicolor</i>	50	100	100
13	<i>Vicia faba</i>	0	0	0
14	<i>Vigna</i>	30	60	100
15	<i>Ricinum communis</i>	0	30	60

The seeds usually start to germinate at 4-6 days after sowing in –contaminated soil (Besalatpour et al., 2008). But there was no germination in the other .Seeds is the most effective step to plant permanence, Which in turn affects the success of the plant treatment process (Frank, 2000).

3.3- Germination of seeds in polluted soil

After examining the vitality of the seeds, the seeds were planted directly in the

contaminated soil with hydrocarbons and compared to seeds germination in clean soil (not contaminated with hydrocarbons).Germination in polluted soil without any modification did not give any results compared to control sample, table (6) and figure (1) show germination result in at 10, 20 and 30 days for contaminated and non contaminated soil.

Table (6) Seeds germination at 10, 20 and 30 days for contaminated and Control (non contaminated) soil

N o.	Seeds plants	Germination%					
		10 Days		20 days		30 Days	
		Contami nated soil	Cont rol soil	Contami nated soil	Cont rol soil	Contami nated soil	Con trol soil
1	<i>Medicago sativa</i>	0	10	0	40	0	50
2	<i>Zea mays</i>	0	0	0	0	0	0
3	<i>Helianthus annuus</i>	0	0	0	0	0	0
4	<i>Trigonella fonom</i>	0	0	0	0	0	0
5	<i>Rhaphanus raphanistrum</i>	0	0	0	0	0	0
6	<i>Phaseolus vulagris</i>	0	0	0	0	0	0
7	<i>Linum</i>	0	10	0	50	0	50
8	<i>Triticum aestivum</i>	0	10	0	50	0	70
9	<i>Hordeum vulgare</i>	0	10	0	0	0	0
10	<i>Sorghum bicolar</i>	0	0	0	0	0	0
11	<i>Dancus carota</i>	0	0	0	0	0	0
12	<i>Red bicolor</i>	0	0	0	0	0	0
13	<i>Vicia faba</i>	0	0	0	0	0	0
14	<i>Vigna</i>	0	0	0	0	0	0
15	<i>Ricinum communis</i>	0	0	0	0	0	0

Contamination soil rustle damage of plants growth ; depending on the degree of contamination .Damage due to soil

contamination may be extensive and its effect may be long term ; it kills plant cells on contact .by kill the roots and this prevents the

plant from absorption water and another nutrients (Torstenssen et al., 1998).

Studies have shown that the plants that are suitable for remediation of a components contaminant should be that can sustain the contaminant (Kirk et al., 2002). Germination

and root extension are two crucial phases in plant growth that are susceptible to ecological contaminants (Band-Grasset et al., 1993). Plants that are able to germinate successfully amidst the contamination and show root elongation are tolerant plant.

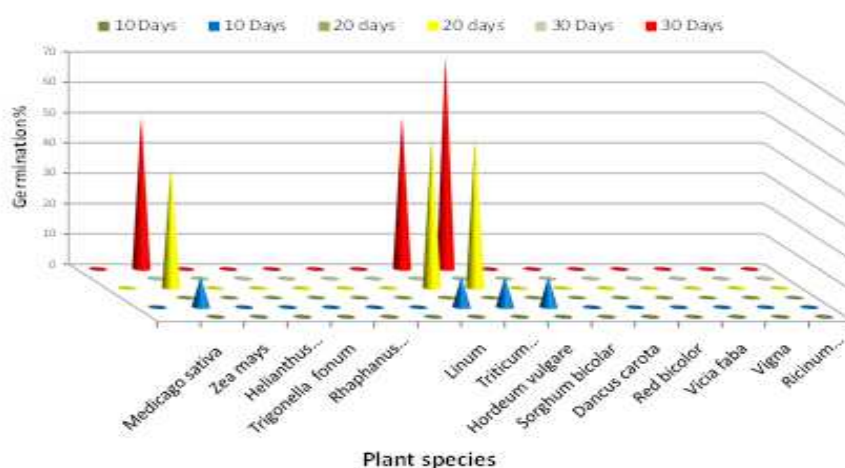


Figure (1) Germination at 10, 20 and 30 days in contaminated and Control (clean soil).

Adam & Duncan (2002) who reported reduction in germination rate in several plant species. The level of contamination determines the extent of damage and also inhibition (Erute et al., 2008). The result in this study agree with the works of (Lessoufi et al., 2006). They recorded reduced seeds germination as the level of contamination increased for plants like *Zea mays*, *Triticum aestivum*, *Medicago sativa*,

Vicia villosa (hairy vetch) and *Glycine max* (Soy bean). (Molina-Barahona et al., 2005) concluded in their study that roots by their direct contact with the polluting agent are the main targets of hydrocarbon in soils and their characteristics play a fundamental role in protection.

3.4- Germination in polluted soil after modification

The results in table (7) and figure (2) showed that after treatment of contaminant soil with hydrocarbons, Germination in all treatment was variation in all treatment.

The soil that used in this study was old and mainly contained large amount of

hydrocarbons ,Thus might be the hydrocarbons is the reasons for lower germination of seeds in the soil. Hydro carbons preventing or lowering entering Oxygen & water to seeds because, may act as physical block a cross out the seeds (Adam & Dunacan, 2002).

Table (7) Germination in all treated soils, + (mean germination), - (non germination)

No.	Plant species	Cod of treated soils							
		A	B	C1	C2	D	E1	E2	F
1	<i>Medicago sativa</i>	-	-	90	-	-	-	-	-
2	<i>Zea mays</i>	-	-	-	-	-	-	-	-
3	<i>Helianthus annuus</i>	-	-	-	-	10	10	10	-
4	<i>Trigonella fonum</i>	-	-	-	-	-	-	-	-
5	<i>Rhaphanus raphanistrum</i>	-	-	-	-	-	-	-	-
6	<i>Phaseolus vulgaris</i>	-	-	-	-	10	-	-	-
7	<i>Linum</i>	-	-	40	-	-	60	-	-
8	<i>Triticum aestivum</i>	-	-	-	50	50	30	-	-
9	<i>Hordeum vulgar</i>	-	-	-	100	100	50	-	-
10	<i>Sorghum bicolar</i>	-	-	10	-	50	70	-	-
11	<i>Dancus carota</i>	-			-	-		-	-
12	<i>Red bicolor</i>	-	-	-	-	40	50	-	-
13	<i>Vicia faba</i>	-	-	-	-	-	-	-	-
14	<i>Vigna</i>	-	-	-	-	-	50	40	-
15	<i>Ricinum communis</i>	-	-	-	20	-	-	-	-

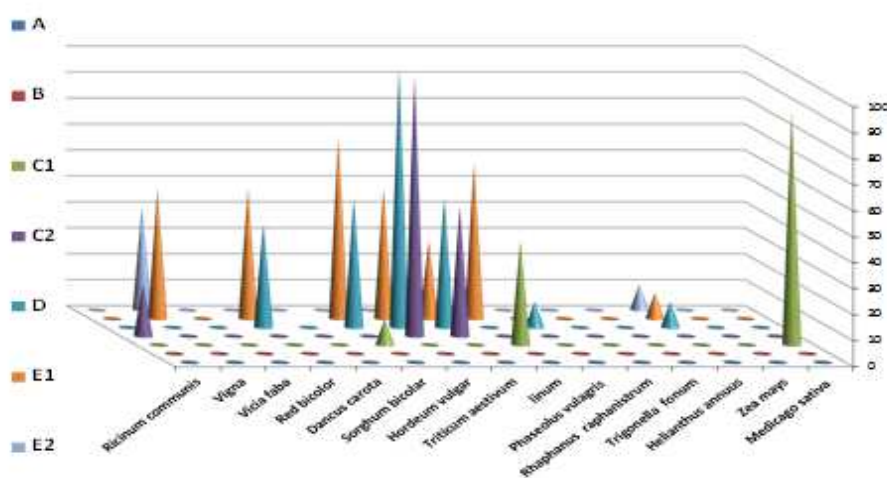


Figure (2) Germination in polluted soil after modification

The results in table (7) and Figure (3) showed the germination was take place in all treatment include treatment A and F not take place any germination for seeds. However, treatment C1 & E1 shows the highest germination in the number of seeds four to five plants. They recorded reduced seed germination in soil processing to 1,2,4,5,6,9,13, and 15 in all processing , Whereas the plant No.3 , 10, and 11 shows germinated in one more than treatment .The presence of hydrocarbons in soil impact germination and then growth of plants in such soil. The impact can be as result of formation

of polar compound melt in water that could intervention the seed coat (Wang et al., 2001).

In treatment E1, that no contain fertilizer was applied, upper germination was recorded .Fertilizer might not have impact on seeds tolerant to petroleum contamination, it sometime possess affirmative impact on plant growth. (Ravan, 2008), even in contaminated soil, by biostimulation. In this study result showed that the impact of animal fertilizer on seeds germination in highly contaminated soil (C1) was very influence that other types of fertilizers used in this study.

Very considerable , the lower germination in soil E2, A , F &C2 was consider to be the

result not only of oil pollution & physical interference of hydrocarbons also fertilizer quality and over fertilizer such as in processes (E2) was probably responsible for the week germination of seeds plant as (Brandt et al., 2006).

On –other hand results showed that seeds plant No. 3, 10, and 11 gave upper rate germination in one more soil treatment that assured that have expansive and intensive root system. With take to it root system and also to its tolerance to high amount petroleum polluted .It seem that seeds plant may be favorable choice for the phytoremediation of TPHs contamination soils.

3.5- Enumerate of Bacteria

Microbial population enhance of plants to degradation of petroleum hydrocarbons in soil in the rhizosphere than are found in the bulk soil (Sicilino et al., 2003). In this study the number of aerobic bacteria in the contaminated soil was (4.2×10^4 CFU/ g soil) figure (3) .the main pathway of phytoremediation is phytostimulation or rhizobiodegradation, in which the remediation of organic pollutants occurs mainly due to the catabolic activities of microorganisms proliferated by the presence of plant roots within the dynamic region of the rhizosphere (Wild 2003 & Sicilino et al., 2003).

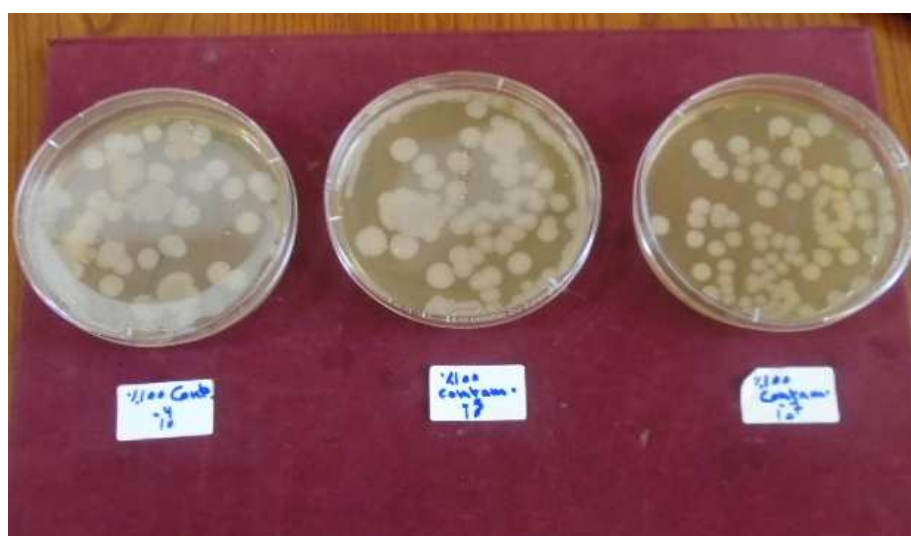


Fig (3) Bactria count in polluted soil with hydrocarbon

3.6- TPH in Soil

After a month of planting the seeds in treated soils, we took the pots where the germination occurred and excluded the pots that did not have any germination process. The concentration of hydrocarbons was measured in the soil samples where the germination

occurred after three month of planting. Will be conducted later in the study. Table (8) and Figure (4) Shows the concentration of hydrocarbons after three month of experimentation, where the initial concentration was 36.3mg / kg in contaminated soil.

Table (8) Concentration of hydrocarbons after three month of experimentation

Plant species	Soil Code	Initial of TPHs g/kg	TPHs after 3 month g/kg	TPHs Removal %
<i>Sorghum bicolor</i>	B	36.3	10.53	71.16
<i>Helianthus annuus</i>	D	36.3	13.12	63.85
<i>Phaseolus vulagris</i>	D	36.3	19.2	47.1
<i>Hordeum vulgare</i>	D	36.3	18.64	48.65
<i>Linum</i>	C2	36.3	18.78	48.2
<i>Hordeum vulgare</i>	C2	36.3	11.46	68.43
<i>Ricinum communis</i>	C2	36.3	22.48	38.07
<i>Linum</i>	E	36.3	12.94	64.35
<i>Hordeum vulgare</i>	E	36.3	13.14	63.8
<i>Helianthus annuus</i>	E	36.3	10.2	71.90
<i>Helianthus annuus</i>	E2	36.3	6.4	82.36

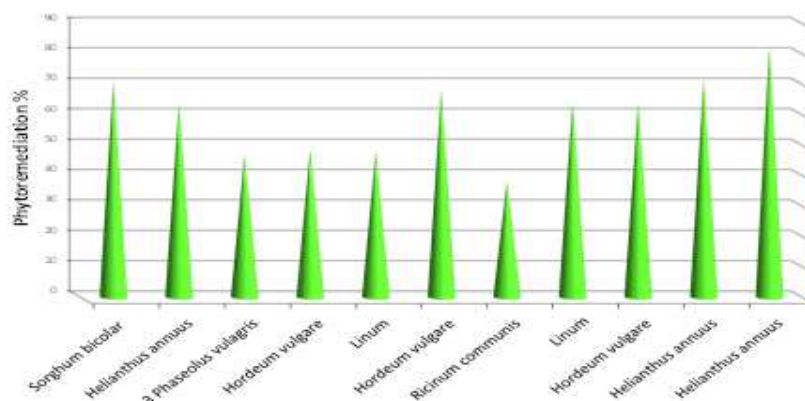


Fig. (4) Concentration of hydrocarbons after three month of experimentation

TPHs reduction was observed in this study after planting for 90 days. During phytoremediation of organic pollutants, plant can immediately take in and convert pollutants, excrete enzymes from root to break down in rhizosphere. Microorganisms decay pollutant or enhance plant growth (Chen et al., 2013).

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للترب الملوثة Phytoremediation دراسة كفاءة إنبات بعض البذور ودورها في المعالجة النباتية بالهيدروكربونات النفطية في محافظة البصرة

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الخلاصة

تم خلال هذه الدراسة اختبار خمسة عشر نوع من البذور جمعت من الأسواق المحلية في مدينة البصرة لتقييم قدرتها على الإنبات والقيام بعملية Phytoremediation للترب الملوثة بالهيدروكربونات النفطية التي جمعت من المناطق القريبة من شركة مصافي الجنوب التي يطلق عليها منطقة الخمسة كيلو في منطقة الشعبية في محافظة البصرة. تم إجراء اختبار الإنبات في طباق بتري قبل الزراعة المباشرة في الترب الملوثة لاختبار كفاءة وحيوية البذور على الإنبات ووضعت في درجة حرارة الغرفة لمدة اسبوعين اذ اظهرت بعض البذور نسبة إنبات اكثر من 95% على مدار اسبوعين. تم زراعة البذور مباشرة في الترب الملوثة في سنادين. اظهرت النتائج ان الإنبات في التربة الملوثة دون أي تعديل لم تعط أي نتيجة مقارنة بعينات السيطرة. نتائج تحسين التربة الملوثة بالهيدروكربونات ببعض الاضافات مثل التخفيف مع التربة النظيفة واطافة السماد الحيواني والبتمس ونشارة الخشب فقد أظهرت معدلات إنبات متفاوتة لأنواع مختلفة من البذور خلال 30 يومًا من التجربه. تم اختيار عدد من النباتات لاستخدامها في التجارب اللاحقة لعملية Phytoremediation تم قياس درجة الحموضة والتوصيل الكهربائي وتركيز المغذيات N.P.K والعدد الكلي للبكتيريا الهوائية. أظهرت نتائج التجربة أن هناك أحد عشر نوعًا من النباتات أعطت نتائج الإنبات في الترب المعاملة، واكثرها كفاءة كانت (*Helianthus anuus*، *Trigonella foenum*، *Medicago sativa*، *Red bicolor and Triticum spelta*) ، كما اظهرت كفاءة على خفض نسبة الهيدروكربونات خلال فترة ثلاثة أشهر من التجربه وسجلت تراكيز الهيدروكربونات الكلية TPHs. يعد إنبات البذور هو الخطوة الأكثر فعالية على انماء النبات ، وهذا بدوره يؤثر على نجاح عملية المعالجة باستخدام النباتات. تم خلال هذا البحث ، دراسة كفاءة بعض بذور النباتات واختيار المناسبة منها لازالة الهيدروكربونات من الترب الملوثة بتقنية المعالجة النباتية.