



Bioremediation of polycyclic aromatic hydrocarbons by using *Zea mays* and inoculating with bacteria (*Pseudomonas aeruginosa*) and fungi (*Penicillium expansum*)

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Absract:

Corn plant (*Zea mays*) was used to remediate polycyclic aromatic hydrocarbons (PAHs) from crude oil polluted soil. The results of physical and chemical analysis of soil revealed that soil was sandy loam, slightly alkaline pH and poor of phosphorus. *Zea mays* plant and its roots associated microorganisms applied to treat polluted soil with crude oil (rhizoremedation) applying augmentation teqnique by inoculating polluted soil with *Pseudomonas aeruginosa* bacteria and *Penicillium expansum* fungi. Total CFU count of bacteria was increased with time while total CFU fungal count was decreased. PAHs were decreased gradually with time, there is a complete removal for many compounds after two months specially when treated with *Pseudomonas aeruginosa* while inoculation with *Penicillium expansum* was less efficient.

Key words: Bioremediation, PAHs, Augmentation, *Zea mays*, *Pseudomonas aeruginosa*, *Penicillium expansum*.

Introduction:

Bioremediation is considering as the best technique for treatment and removal of contaminants and resulting into less hazardous substances. It depends on the ability of microorganisms to degrade complex pollutants into less hazardous, harmless substances, which are then enter into

natural biogeochemical cycles. It has been applied in the most contaminated sites all over the world [1]. Microbes which are present in the rhizosphere region of plant cultivated soil have the basic role in phytoremediation, therefore the plant's root system is one of the most important factors [2]. The combination of phytoremediation and microbial remediation has become a general practice in the field of treatment of crude oil contaminated sites. Plants also can influence degradation indirectly by changing the chemical and physical conditions of the soil [3].

Crude oil hydrocarbons are used as a source of energy for microbial survive, and at the same time, microorganisms metabolize them to many substances include: naphthenic acids, phenols, alcohols, hydroperoxides, esters, carbonyl compounds, and finally to CO₂ and H₂O [4]. This is resulted from the role of fungi, bacteria and algae which secretes enzymes able of breakdown hazardous organic contaminants [5].

Health risks results from the direct contact with the oil polluted soil, vapors emits from contaminants, and from secondary pollution of underground water which underlying the contaminated soil. The seepage of crude oil through the soil damaging the biological communities in the soil, including plants and microorganisms [6].

PAHs are class of petroleum organic compounds that consist of two or more fused benzene rings and/or pentacyclic molecules which are arranged in different structural configurations. PAHs result primarily from combustion of fuel with high boiling points, very low water solubility, high toxicity and stability. PAHs are poorly soluble, hydrophobic organic compounds results from the combustion of organic materials, releasing to the environment as a result of anthropogenic activity as well as from natural geological processes. PAHs considered as an important class of contaminants because they are among the most frequent compounds found in contaminated soil [7,8].

PAHs ubiquity in addition to their mutagenicity and toxicity, makes them priority contaminants, the US Environmental Protection Agency (EPA) listed sixteen PAHs as priority contaminants [9]. The persistent of high molecular weight hydrocarbons (polycyclic aromatic hydrocarbon with three and more rings) is because of their low water solubility and low volatility and will remain in the environment for very long time. While low molecular weight aromatic hydrocarbons (one or two rings) have a rapid volatilize rate. [10].

The current study investigated the remediation of PAHs from crude oil polluted soil by cultivating *Zea mays* plant and applying augmentation technique by using bacteria (*Pseudomonas aeruginosa*) and fungi (*Penicillium expansum*).

Materials and methods:

Uncontaminated soil was collected from Al-Tajiyah region, Hilla city, Babylon Province, Iraq, was taken from the upper layer (25-30 cm in depth) of the soil, dried by air and sieved. Medium crude oil was obtained from Al-Najaf Oil Refinery, 75 gm of crude oil has been added to each kg of uncontaminated soil, mixed very well and let for two weeks to dry by air to allow volatilization of volatile compounds. After that, pots were underlined with aluminum foil. 5 kg of oil contaminated soil were put in each pot, all pots were firstly watered to full extent with water and then laid for 3 days in

order to make fully blended of the petroleum, soil and water to reach stable state [11]. Physical and chemical properties of soil were measured three times: at the beginning of the experiment, after one month and after two months at the end of the experiment.

Temperature was measured by celosias thermometer. Electrical conductivity was measured by E.C meter (Hanna / 214) in the soil extract 1:1. Soil texture and total phosphorus were measured by method of [12]. Also Moisture, pH and salinity were measured [13].

Experimental design:

Experiments were involved cultivated polluted soil for two months from April to May, 2016. Sampling was each two weeks. The treatments with three replicates as follows:

1. Unpolluted soil (control) cultivated with *Z. mays*.
2. Crude oil polluted soil cultivated with *Z. mays*.
3. Crude oil polluted soil cultivated with *Z. mays* inoculated with *Pseudomonas aeruginosa*.
4. Crude oil polluted soil cultivated with *Z. mays* inoculated with *Penicillium expansum*.
5. Crude oil polluted soil cultivated with *Z. mays* inoculated with combination of *Pseudomonas aeruginosa* and *Penicillium expansum*.

Inoculation of soil and microbial population count:

Pseudomonas aeruginosa and *Penicillium expansum* were isolated from Al-Najaf Oil Refinery polluted soil, they were the most dominant isolated species of bacteria and fungi in crude oil polluted soil of the refinery which can live and utilize hydrocarbons. *P. aeruginosa* was inoculated into nutrient broth and incubated at 37° C. The bacterial count was carried out by measuring absorbance using spectrophotometer at an absorbance of 560 nm wavelength, until a cell concentration of 1.5×10^8 colony forming units (CFU)/ml (1 McFarland Standard) was achieved [14].

P. expansum inoculum was prepared according to [15] by removing spores from the surface of potato dextrose agar with a sterilized needle to be suspended in normal saline, then filtrate or centrifuged and using direct method to calculate the spores concentration using haemocytometer and applying the equation:

$$\text{No. of spores/ml} = \text{average of spores number in four sq.} \times 10^4$$

The samples were processed using soil dilution plate method, dilution was up to 10^{10} and then 0.1 ml of dilution was added to 20 ml of nutrient agar medium for bacteria and potato dextrose agar for fungi, in 90 mm diameter sterile Petri dishes.

Soil samples after serial dilution plates were incubated to 48 hours to grow the bacterial colonies properly and 7 days for fungi, then enumerated. Colony forming units (CFU) were counted by using a colony counter [14], then applying the following equation:

$$\text{cfu / ml} = \text{number of colonies} \times \frac{1}{\text{dilution factor}} \times \text{plating factor}$$

Extraction of PAHs from soil:

In this study, extraction was conducted by ultrasonication method using ultrasonic instrument. 1 gm of contaminated soil was dried at room temperature and after sieving (using sieve #50), was suspended in 10 mL of acetonitrile and extracted by ultrasonic bath at 40-45 °C for 60 minutes. Extracts were settled for 10 minutes and centrifuged at 6000 rpm for 15 minutes [16].

Gas Chromatography (GC) analysis:

Gas Chromatography technique with Flame Ionization Detector (FID) to measure extracted PAHs from plants and soils. Standard solution was contain twelve (PAHs) compounds they are: Naphthalene, Tetraphan, Acenaphthylene, Flourene, Phenanthrene, Anthracene, Pyrene, Benz (a) anthracene, Ovalene, Chrysene, Benz (b) fluoranthen and Dibenz (ah) anthracene. The concentrations were calculated according to the equation:

$$Cs = \frac{CSt \times As}{Ast}$$

Cs = Concentration of sample

Cst = Concentration of standard

As = Area of sample

Ast = Area of standard

Results and Discussion:

Physical and chemical properties of soil:

Results of physical and chemical properties of soil revealed that soil is sandy loam composed of clay 28%, silt 32% and sand 40%, and it is slight alkaline. Properties were measured three times through experiments, the first measurement was at the beginning of the experiment at 25°C, the second measurement was after one month at 29°C and at the end of the experiment after two months at 43°C. The measured properties were pH, salinity, moisture, and total phosphorus.

The values of measured properties decreased gradually from the beginning of the experiment until the end of the experiment for all measured parameters of soil except of temperature and moisture content were increased with time. Results shows there is a significant decrease of moisture content and a significant increase of pH in comparison with unpolluted soil at the beginning of the experiment (table 1). The second measurement was after one month, the results showed also a significant increase of pH, salinity, and a significant decrease of moisture in comparison with unpolluted soil (table 2). While at the end of the experiment results indicated that there is a significant difference between unpolluted soil and polluted soil for salinity and moisture table (3).

Table 1: physical and chemical properties of soil at the beginning of the experiment at 25°C

No.	Treatments	pH	Salinity ‰	Moisture %	Total Phosphorus%
1	polluted soil + <i>Z. mays</i> (control)	7.73± 0.06	1000.± 0.31	19.14± 0.15	Nil
2	polluted soil + <i>Z. mays</i>	7.93± 0.06	1000± 0.23	10.33± 0.12	Nil
3	polluted soil + <i>Z. mays</i> + <i>P. aeruginosa</i>	7.96± 0.06	1000± 0.12	9.59± 0.54	Nil
4	polluted soil + <i>Z. mays</i> + <i>P. expansum</i>	8.03± 0.06	1000± 0.1	9.83± 0.16	Nil
5	polluted soil + <i>Z. mays</i> + <i>P. aeruginosa</i> + <i>P. expansum</i>	8.00± 0.1	1000.1 ±0.12	9.42± 0.61	Nil
6	LSD (0.05)	0.1	N.S	0.56	N.S

* Each value represents mean ± standard deviation.

* Nil = negligible value.

* N.S = non-significant difference.

Table 2: physical and chemical properties of soil after one month at 29°C.

No.	Treatments	pH	Salinity ‰	Moisture %	Total Phosphorus%
1	polluted soil + <i>Z. mays</i> (control)	7.70 ±0.1	701 ±12	20.10 ±0.4	Nil
2	polluted soil + <i>Z. mays</i>	7.8 ± 0.05	660.6 ±38	12.57 ±0.5	Nil
3	polluted soil + <i>Z. mays</i> + <i>P. aeruginosa</i>	7.8 ±0.05	620.6 ±18	16.89 ±0.29	Nil
4	polluted soil + <i>Z. mays</i> + <i>P. expansum</i>	7.9 ±0.05	664.3 ±26	13.15 ±0.33	Nil
5	polluted soil + <i>Z. mays</i> + <i>P. aeruginosa</i> + <i>P. expansum</i>	7.90 ±0.1	600.6 ±37	17.13 ±0.69	Nil
6	LSD (0.05)	0.11	42.55	0.7	N.S

* Each value represents mean ± standard deviation.

* Nil = negligible value.

* N.S = non-significant difference.

Table 3: physical and chemical properties of soil after two months at 43°C

No.	Treatments	pH	Salinity ‰	Moisture %	Total Phosphorus %
1	polluted soil + <i>Z. mays</i> (control)	7.60 ±0.1	315.33 ±6.5	21.08 ±1.3	Nil
2	polluted soil + <i>Z. mays</i>	7.76 ±0.05	434.67 ±22.7	17.01 ±0.5	Nil
3	polluted soil + <i>Z. mays</i> + <i>P. aeruginosa</i>	7.86 ±0.05	392.00 ±16	18.75 ±0.56	Nil
4	polluted soil + <i>Z. mays</i> + <i>P. expansum</i>	7.83 ±0.05	409.00 ±29	18.12 ±0.18	Nil
5	polluted soil + <i>Z. mays</i> + <i>P. aeruginosa</i> + <i>P. expansum</i>	7.83 ±0.05	327.00 ±19.6	19.82 ±0.53	Nil
6	LSD (0.05)	0.12	30.14	1.07	N.S

* Each value represents mean ± standard deviation.

* Nil = negligible value.

* N.S = non-significant difference.

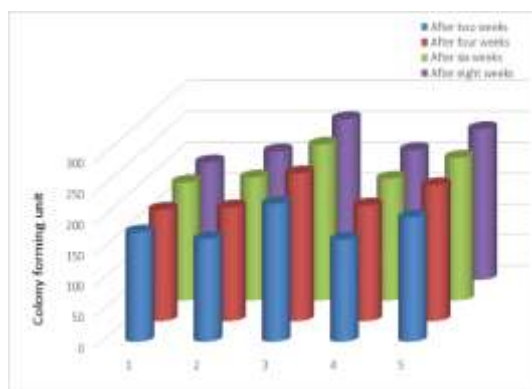
Plant life was effected negatively with petroleum pollution, because of the changes of physical and chemical properties of petroleum polluted soil in comparison with unpolluted soil [19]. Soil texture as an important factor because its relation with soil microorganisms; the amount and type of clay which is present in the soil can affect the soil matrix, and therefore, microorganisms presence and activity. When [18] examined the effect of crude oil spills on soil chemical properties, results of high concentration of petroleum was indicated that phosphorus decreased in the soil.

Also pH could affects the growth of plants as a result of changing the availability of nutrients. Elevated or low values of pH could cause deficiencies of essential nutrients for growth of plant. Increased soil pH after pollution could be attributed to microorganism's anaerobic breakdown of petroleum in soil, resulting in raise of soil pH. As a result of released CO₂ which contributed to the alkalinity in the treated soil [19]. Also oil could decrease soil pH as a result of releasing organic matter by microorganisms metabolic processes [20].

Salinity was decreased with time passage, it is effecting on phytoremediation rate as mentioned by [21] the biodegradation rate will be decreased if soil salinity increased more than an optimum level. While soil moisture decreased with oil pollution and could affect the biodegradation ratio by effecting on the bioavailability of hydrocarbons, transport of produced gases, diffusion processes, oxygen availability in the soil and soil toxicity level [22]. Soil was poor of phosphorus; alkaline soils usually have negligible values of phosphorus [23].

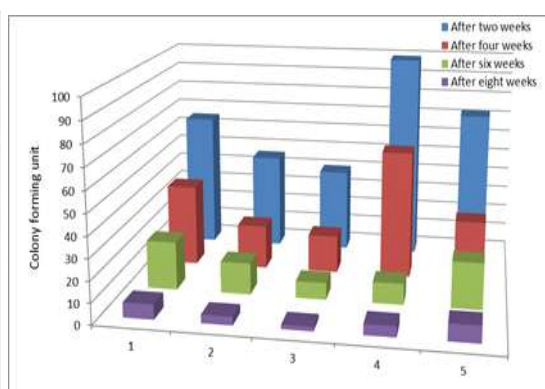
Total bacterial and fungal count:

Total bacterial and fungal counts of crude oil polluted soil were did each two weeks, total counts of bacteria were decreased significantly at the non-inoculated polluted soil and inoculated with fungi in comparison with total bacterial count of unpolluted cultivated soil (control), while the total count increased significantly at the inoculated soil with bacteria and inoculated with bacteria and fungi (figure 1). While total fungal counts were decreased significantly at polluted non-inoculated soil, inoculated with bacteria and inoculated with bacteria and fungi in comparison with total fungal count of cultivated unpolluted non-inoculated soil (control), the significant lowest value was for inoculated with bacteria. While the count of fungi inoculated soil was increased significantly (figure 2).



LSD treatment = 2.87, LSD time = 2.57 $p \geq 0.05$

Figure 1: total bacterial count $\times 10^9$ cfu/ml of the treatments (1: unpolluted soil, 2: untreated polluted soil, 3: polluted soil treated with *Pseudomonas aeruginosa*, 4: polluted soil treated with *Penicillium expansum*, 5: polluted soil treated with *Pseudomonas aeruginosa* and *Penicillium expansum*) during two months.



LSD treatment = 2.15, LSD time = 1.92 $p \geq 0.05$

Figure 2 (reversed): total fungal count $\times 10^9$ cfu/ml of the treatments (1: unpolluted soil, 2: untreated polluted soil, 3: polluted soil treated with *Pseudomonas aeruginosa*, 4: polluted soil treated with *Penicillium expansum*, 5: polluted soil treated with *Pseudomonas aeruginosa* and *Penicillium expansum*) during two months.

Total CFU bacterial count of rhizosphere of petroleum polluted soil revealed a significant decrease of total counts of polluted untreated soil compared with unpolluted soil. These results are in line with [18] who polluted soil with 75 gm/kg of crude oil, she mentioned that the total CFU bacterial counts of crude oil polluted soil was less than that of control (uncontaminated). If petroleum contacted with soil it will cause damage of cultivated plants and agricultural lands with their microorganisms [19]. Microorganisms will be effected by cell membrane permeability reduction, entirely blocking or decreasing the capacity of taking elements from soil resulted in their starvation and death. Microorganisms also will be effected by the direct exposure to the toxic chemicals which inhibiting their growth [20].

Total CFU bacterial count is effected by soil properties and by the type of hydrocarbons. Texture of soil represents an important factor that effecting microorganism's life, clay and organic matter will stop the negative effects of crude oil on soil microorganisms. High clay content and organic matter make petroleum tend to sorption and decreasing its bioavailability which lead to high CFU total count compared with sandy soil [21], while the soil texture was sandy loam (28% for clay),.

Another factor is soil pH which effecting microorganisms diversity. High heterotrophic total bacterial CFU count are related with low pH values [22], compared with elevated pH results of this study was $pH \geq 7.7$. Total CFU bacterial count were increased with the passage of time, this is as a result of increased moisture content and temperature. Temperature effecting rates of metabolic processes, therefore the rates of many reactions are doubled for each 10 °C elevation of temperature, also moisture content is necessary for microorganisms [23].

Inoculation with *Pseudomonas aeruginosa* (bioaugmentation) showed a higher total bacterial counts than uncontaminated soil as a result of addition of inoculum and the ability of this bacteria to grow in petroleum polluted soil. Native or indigenous microorganisms are usually presents with little quantities which can't control the spread of contamination because they can't degrade a particular contaminant, for this reason the bioaugmentation is more favor than biostimulation [٣١]. *Pseudomonas aeruginosa* can live and grow in high concentrations of hydrocarbons (up to 50% v/v) and using hydrocarbons as a source of energy, the ability of *Pseudomonas aeruginosa* to breakdown hydrocarbons make it the most active utilizer of hydrocarbons [3].

Results of total CFU fungal counts of oil contaminated soil were decreased significantly with time even when inoculated with *Penicillium expansum* the lower significant value was of soil which inoculated with bacteria. This is in line with the results of [3١] study who studied the capacity *Aspergillus* and *Penicillium* species at acidic and alkaline soil pH, he reported that the lowest growth of *Aspergillus* was at alkaline pH (8 - 8.5), while *Aspergillus* was predominant and *Penicillium* was not observed. Fungal populations were high at low soil pH =5.5, the pH of the studied soil was alkaline which is not suitable for many fungal species including *Penicillium*.

Total CFU counts were effected negatively with increase of temperature. [٣2] mentioned that microorganisms growth live in temperature ranges between 25 and 30 C, when temperature elevated total counts were decreased. Fungi need to live in low temperature therefore would be effected more than bacteria, the result from that is decreased total CFU fungal count. Also decreased total CFU fungal count of polluted soil inoculated with *Pseudomonas* is relating to the antagonism relationship between *Pseudomonas* bacteria and *Penicillium expansum*. This was reported by [٣3] when used *Pseudomonas* control *Penicillium expansum*, *Pseudomonas* decreased fungal growth to 78.5%.

Polycyclic Aromatic Hydrocarbons (PAHs):

Twelve of polycyclic aromatic hydrocarbons were measured each two weeks for two months, results showed a complete removal of Benz (a) anthracene, Chrysene, Benzo (b) fluoranthene and Dibenzo (ah) anthracene after 14 days of the experiment. Graduated decrease was observed for Naphthalene, Tetraphan, Acenaphthylene, Flourene, Phenanthrene, Anthracene, Pyrene and Ovalene when measured each two weeks.

Results revealed that PAHs removal increased with increased temperature and time. Also the treatments with bacteria and the combination between bacteria and fungi are more effective than treatment with fungi in comparison with polluted untreated soil at the same time when measured.

After eight weeks results showed there is a complete removal of Tetraphan, Acenaphthylene, Flourene, Anthracene, Pyrene and Ovalene in addition to those removed after two weeks. While the residual concentrations of other compounds was greatly decreased in comparison with the concentrations of the first two weeks table (4), (5), (6) and (7).

Table 4: PAHs concentrations (mg/gm) in crude oil polluted soil
cultivated with *Zea mays* after two weeks at 25° c

No.	PAHs	Unpolluted soil + plant	Polluted untreated soil	Polluted soil + plant	Polluted soil + plant +bacteria	Polluted soil + plant +fungi	Polluted soil + plant +bacteria +fungi
1	Naphthalene	N.D	1135	23.8	15.54	27.95	13.79
2	Tetraphan	N.D	550	108.5	17.3	19.59	10.64
3	Acenaphthylene	N.D	27	N.D	N.D	N.D	8.43
4	Flourene	N.D	358	17.3	9.58	11.45	5
5	Phenanthrene	N.D	167	32.99	7.2	4.93	9.83
6	Anthracene	N.D	7.5	N.D	N.D	N.D	0.67
7	Pyrene	N.D	6.22	3.94	N.D	0.56	0.13
8	Benz (a) anthracene	N.D	N.D	N.D	N.D	N.D	N.D
9	ovalene	N.D	63	47.11	9.89	17.43	8.2
10	chrysene	N.D	23.5	N.D	3.22	N.D	N.D
11	Benzo (b) fluoranthene	N.D	2.7	N.D	N.D	N.D	N.D
12	Dibenz (ah) anthracene	N.D	N.D	N.D	N.D	N.D	N.D
13	Total PAHs	N.D	2339.92	233.64	62.73	81.91	56.69

*N.D = Not Detected

Table 5: PAHs concentrations (mg/gm) in crude oil polluted soil
cultivated with *Zea mays* after four weeks at 29° c

No.	PAHs	Unpolluted soil + plant	Polluted untreated soil	Polluted soil + plant	Polluted soil + plant +bacteria	Polluted soil + plant +fungi	Polluted soil + plant +bacteria +fungi
1	Naphthalene	N.D	1135	11.6	8.31	18.3	7
2	Tetraphan	N.D	550	8.92	9.6	13.1	6.41
3	Acenaphthylene	N.D	27	N.D	N.D	N.D	5.31
4	Flourene	N.D	358	3.52	2.22	7.09	3.41
5	Phenanthrene	N.D	167	8.48	N.D	N.D	1.8
6	Anthracene	N.D	7.5	N.D	N.D	N.D	N.D
7	Pyrene	N.D	6.22	0.9	N.D	N.D	N.D
8	Benz (a) anthracene	N.D	N.D	N.D	N.D	N.D	N.D
9	ovalene	N.D	63	18.27	6.49	N.D	5.2
10	chrysene	N.D	23.5	N.D	N.D	N.D	N.D
11	Benzo (b) fluoranthene	N.D	2.7	N.D	N.D	N.D	N.D
12	Dibenz (ah) anthracene	N.D	N.D	N.D	N.D	N.D	N.D
13	Total PAHs	N.D	2339.92	51.69	26.62	38.49	29.13

*N.D = Not Detected

Table 6: PAHs concentrations (mg/gm) in crude oil polluted soil cultivated with *Zea mays* after six weeks at 37° c

No.	PAHs	Unpolluted soil + plant	Polluted untreated soil	Polluted soil + plant	Polluted soil + plant +bacteria	Polluted soil + plant +fungi	Polluted soil + plant +bacteria +fungi
1	Naphthalene	N.D	1135	6.4	N.D	5.2	N.D
2	Tetraphan	N.D	550	6.2	N.D	5.75	N.D
3	Acenaphthylene	N.D	27	N.D	N.D	N.D	N.D
4	Flourene	N.D	358	3.3	N.D	2.41	N.D
5	Phenanthrene	N.D	167	2.9	1.48	N.D	1.67
6	Anthracene	N.D	7.5	N.D	N.D	N.D	N.D
7	Pyrene	N.D	6.22	0.4	N.D	N.D	N.D
8	Benz (a) anthracene	N.D	N.D	N.D	N.D	N.D	N.D
9	ovalene	N.D	63	7.5	N.D	N.D	3.6
10	chrysene	N.D	23.5	N.D	N.D	N.D	N.D
11	Benzo (b) fluoranthene	N.D	2.7	N.D	N.D	N.D	N.D
12	Dibenz (ah) anthracene	N.D	N.D	N.D	N.D	N.D	N.D
13	Total PAHs	N.D	2339.92	26.7	1.48	13.36	5.27

*N.D = Not Detected

Table 7: PAHs concentrations (mg/gm) in crude oil polluted soil cultivated with *Zea mays* after eight weeks at 43° c

No.	PAHs	Unpolluted soil + plant	Polluted untreated soil	Polluted soil + plant	Polluted soil + plant +bacteria	Polluted soil + plant +fungi	Polluted soil + plant +bacteria +fungi
1	Naphthalene	N.D	1135	N.D	N.D	1.04	N.D
2	Tetraphan	N.D	550	N.D	N.D	N.D	N.D
3	Acenaphthylene	N.D	27	N.D	N.D	N.D	N.D
4	Flourene	N.D	358	N.D	N.D	N.D	N.D
5	Phenanthrene	N.D	167	1.29	N.D	N.D	N.D
6	Anthracene	N.D	7.5	N.D	N.D	N.D	N.D
7	Pyrene	N.D	6.22	N.D	N.D	N.D	N.D
8	Benz (a) anthracene	N.D	N.D	N.D	N.D	N.D	N.D
9	ovalene	N.D	63	N.D	N.D	N.D	N.D
10	chrysene	N.D	23.5	N.D	N.D	N.D	N.D
11	Benzo (b) fluoranthene	N.D	2.7	N.D	N.D	N.D	N.D
12	Dibenz (ah) anthracene	N.D	N.D	N.D	N.D	N.D	N.D
13	Total PAHs	N.D	2339.92	1.29	N.D	1.04	N.D

*N.D = Not Detected

Petroleum oil consists of various hydrocarbons involves more than 30 polycyclic aromatic hydrocarbons (PAHs) [34]. PAHs are polycyclic aromatic compounds containing two or more than to eight fused rings. There are 30 PAHs compounds and different derivatives had identified as a carcinogenic and mutagenic compounds, considering them the largest class of chemical carcinogenic substances [35]. PAHs represents a major hazards because of their carcinogenicity and mutagenicity, persistence, and the presence of these components in food represents a threat to human life [36]. The EPA has classified the following PAH compounds: benz (a) anthracene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (k) fluoranthene, chrysene, dibenz (ah) anthracene, and indeno(1,2,3-cd) pyrene as carcinogens [37]. This study involved four compounds of these seven carcinogens.

Many polycyclic aromatic hydrocarbons had been identified as a cause of cancer in large number of vertebral animals, including human, in addition to many invertebrates. Researchers found that increased cases of lung cancer results from the inhalation of PAHs which are emits from combustion of organic materials [38]. They causing several diseases to human for example Phenanthrene causing skin mild allergy and require rapid remediation [39]. PAHs are high soluble in lipids and therefore will be easily absorbed in the mammals gastrointestinal tract. PAHs are distributed rapidly in various body tissues with a high tendency to localize adipose tissues [40]. Therefore bioremediation and biodegradation became an important approach to remove PAHs from polluted environments [41].

The recognition among various PAHs are based on their molecular weight, and their hydrophobicity, their tendency of accumulation, their resistance to degradation, therefore their persistence usually increasing with the increasing of molecular weight. Low molecular weight (LPAHs) such as Naphthalene, Acenaphthylene, Fluorene, Anthracene and Phenanthrene reported as more acute toxic to living organisms than High molecular weight (HPAHs) such as Fluoranthene, Pyrene, Benz(a)anthracene, Chrysene, Benzo(b)fluoranthene, pyrene, Dibenz(a,h)anthracene and Ovalene, since they are more water-soluble. High molecular weight PAHs are tending to posses molecular structures of four or more benzenoid rings, and including fluoranthene, pyrene, and benzo(b)fluoranthenes [42].

Results showed that there is a complete removal of Chrysene and Benzo (b) fluoranthene after two weeks of contamination. The high residual concentrations was for Naphthalene, Tetraphan, Phenanthrene and Ovalene.

Although low molecular weights compounds are with high concentrations but results indicated that these compounds were highly reduced after two months from pollution in comparison with high molecular weights compounds because low molecular weight PAHs are tending to be more volatile than high molecular weight PAHs [43].

This study revealed there is a slow rate removal of PAHs from untreated cultivated oil polluted soil in comparison with treated cultivated polluted soil. [44] recorded that the crude oil can't persist for long time in polluted soils even when high amounts of oil had spilled, as a result of the initial

degradation by the role of temperature in volatilization, effects of sunlight by photolysis followed by endogenous microbial degradation when the petroleum sinks.

Treatments of cultivated polluted soil with bacteria and combination between bacteria and fungi were the most effective than treatment with fungi in comparison with cultivated untreated polluted soil. Based on the ability of degrading petroleum, *Pseudomonas* is the most efficient crude oil hydrocarbon degrader. Many observations had reported that the *Pseudomonas* genus is the most efficient degrader among other hydrocarbon degrading microorganisms [14].

Results indicated that bioremediation enhanced with elevated temperature, that is evaporation is another mechanism through which high temperature increase the biodegradation rate. The evaporation rate of low molecular-weight hydrocarbons drops off with temperature [15].

Also results showed that high molecular weight compounds were degraded with lower rate than that of low molecular weight. [16] studied the aging of PAHs in the environment, they found that half-life of PAHs compounds increase with increased rings number and molecular weight of each compound, suggesting that more than three rings compounds needs more time to removal.

Conclusion:

Bioremediation of polycyclic aromatic hydrocarbons by using *Zea mays* plant from crude oil polluted soil is efficient. There is a complete removal of many polycyclic aromatic hydrocarbons after two months of the experiment specially when treated with *Pseudomonas aeruginosa* bacteria.

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