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Bioremediation of polycyclic aromatic hydrocarbons polluted soils using augmentation by inoculating with bacteria (*Pseudomonas aeruginosa*) and fungi (*Penicillium expansum*

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Abstract:

Crude oil polluted soil was treated with bacteria *Pseudomonas aeruginosa* and fungi *Penicillium expansum*. The results of physical and chemical analysis of soil revealed that soil was sandy loam, slightly alkaline pH, moisture content of polluted soil was less than that of unpolluted soil. Augmentation teqnique was applied by inoculating polluted soil with *Pseudomonas aeruginosa* bacteria and *Penicillium expansum* fungi. Results revealed that total CFU count of bacteria was increased while total CFU fungal count was decreased after two months. PAHs concentrations were decreased gradually during two months, results showed a complete removal of many compounds after the treatment with bacteria while fungi was not effective.

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

Introdution:

Soil pollution results from the continuous development of the economy of the world, pollution with oil compounds became a global concern because of using petroleum products as the main source of energy [1]. The accumulation of oil hydrocarbons in the soil will cause hazard problems, change the nature of ecosystems and can also cause health hazards for human and animals [2]. Oil pollution can change the physical, chemical and biological characteristics of the soil, then changing nutrient concentrations which need for plant growth, these negative effects are including the reduced productivity of the cultivated soils, which lead to damage economy and biodiversity in the regions that depend on agriculture, causing high poverty rate (3). Polycyclic aromatic hydrocarbons (PAHs) are mutagenic and toxic pollutants and Environmental Protection Agency (EPA) listed 16 of PAHs as priority pollutants [4]. Bioremediation (a green technology) leads to pollutants degradation, it's a lucrative and environmentally beneficial alternative which considered as a source of economic profit [5]. It is a process depending on the abilities of soil microorganisms to increase the rate of pollutant degradation, it is an important process to remove soil pollution, the rate of destruction of organic compounds will be dependent on the structure of these compounds, the microbial community, the type and level of pollution [6]. Dstruction of hydrocarbons by microbes is a very slow process usually, but it may be optimum degradation under a certain environmental circumstances such as temperature, pH, nutrients, soil texture, oxidation-reduction potential and mixture of microbial consortia which are present [7]. The effective bioremediation depends on the extent to which a consortium can be maintained in environment, microbial destruction can be achieved by several microbial species which are native or added to the polluted soil (bioaugmentation) as efficient strains [8].

Materials and methods:

Unpolluted soil was taken from Al-Tajiyah region, Hilla city, Babylon Province, Iraq, from the upper layer (25-30 cm in depth) of the soil, left to dry by air and then sieved. Medium crude oil was obtained from Al-Najaf Oil Refinery, 75 gm of crude oil has been mixed with each kg of unpolluted soil very well and left for two weeks to dry by air temperature and to allow volatile compounds to be volatilized, pots were underlined with aluminum foil. 5 kg of crude oil polluted 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 [9]. Physical and chemical properties of pots 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. Soil texture, Moisture, potassium and bicarbonates measured by methods of [10]. Also Salinity and pH measured by E.C multiple meter (Hanna / 214) in the soil extract 1:1 [11].

Experimental design:

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

1. Unpolluted soil (control).
2. Crude oil polluted soil.
3. Crude oil polluted soil inoculated with *Pseudomonas aeruginosa.*

4. Crude oil polluted soil inoculated with *Penicillium expansum.*

5. Crude oil polluted soil 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 breakdown hydrocarbons. Nutrient broth of *P. aeruginosa* incubated at 37^{o} C for 24 hours and the bacterial count carried out by measuring absorbance using spectrophotometer at an absorbance of 560 nm wavelength, until achieve cell concentration of 1.5 * 108 colony forming units (CFU)/ml (1 McFarland Standard) (12). Inoculum of *P. expansum* inoculum prepared as mentioned in [13] by collecting 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 following equation:

No. of spores/ml = average of spores number in four sq. * 104

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:

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

Extraction of PAHs from soil:

Extraction of PAH conducted by ultrasonication method using ultrasonic water bath. 1 gm of polluted soil dried at room temperature and sieved later (using sieve #50), suspended in 10 mL of acetonitrile and extracted by ultrasonic bath at 40-45 ^{o}C for 60 minutes. Extracts were settled for 10 minutes and centrifuged at 6000 rpm for 15 minutes [15].

Gas Chromatography (GC) analysis:

Gas Chromatography teqnique with Flame Ionization Detector (FID) was used 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 showed that soil is sandy loam composed of clay 28%, silt 32% and sand 40%, and it is slight alkaline. The measurements involved pH, salinity, moisture, potassium and bicarbonates. Measurements were achieved three times through the period of the experiments, the first measurement was at the beginning of the experiment at 25℃, the second measurement was after one month at 29℃ and at the end of the experiment after two months at 43℃.

The results showed a graduated decrement from the beginning until the end of the experiments for all measured properties of soil except of temperature and moisture contents were increased gradually 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 revealed that there is a significant differences between unpolluted and polluted soil for salinity and moisture (table 3).

Table 1: Physical and chemical properties of crude oil polluted soil at the beginning of the experiment at 25° C

No.	Treatments	pH	Salinity dS/m	Moisture content %	Potassium Mg/gm	Bicarbonates meq/kg
1	Unpolluted soil (control)	7.96 ±0.05 **a**	1000 ±0.11 **a**	20.90 ±0.52 **b**	11.26 ±0.37 **a**	9.00 ±0.45 **a**
2	Polluted soil	8.03 ±0.05 **a**	1000.9 ±0.17 **a**	10.01 ±0.08 **a**	11.06 ±0.2 **a**	9.03 ±0.49 a
3	Polluted soil + bacteria	7.96 ±0.05 **a**	1000.3 ±0.57 **a**	10.04 ±0.19 **a**	10.96 ±0.37 **a**	8.80 ±0.36
4	Polluted soil + fungi	8.03 ±0.05 **a**	1000.4 ±0.52 **a**	9.93 ±0.06 **a**	11.00 ±0.5 **a**	8.70 ±0.3 **a**
5	Polluted soil + bacteria +fungi	7.96 ±0.05 **a**	1000.5 ±0.5 **a**	10.03 ±0.3 **a**	10.86 ±0.15 **a**	8.60 ±0.52 **a**

* Each value represents mean ± standard deviation.
* Nill = negligible value.
* The similar litters of each parameter refers to non-significant difference $p > 0.05$.

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

No.	Treatments	pH	Salinity dS/m	Moisture content %	Potassium Mg/gm	Bicarbonates meq/kg
1	Unpolluted soil (control)	7.93 ±0.05 **a**	993.90 ±5.4 **a**	20.13 ±0.41 **d**	9.33 ±0.2 **a**	7.00 ±0.36 **a**
2	Polluted soil	7.96 ±0.05 **a**	998.67 ±2.3 **a**	11.06 ±0.38 **ab**	10.26 ±0.2 **c**	8.20 ±0.3 **b**
3	Polluted soil + bacteria	7.93 ±0.05 **a**	993.00 ±9.64 **a**	11.52 ±0.26 **bc**	9.63 ±0.3 **ab**	7.53 ±0.45 **a**
4	Polluted soil + fungi	7.96 ±0.05 **a**	982.33 ±23.24 **a**	10.50 ±0.36 **a**	10.00 ±0.3 **bc**	8.20 ±0.1 **b**
5	Polluted soil + bacteria +fungi	7.96 ±0.05 **a**	1000.5 ±0.5 **a**	11.93 ±0.53 **c**	9.26 ±0.3 **a**	7.40 ±0.17 **a**

* Each value represents mean ± standard deviation.

* Nill = negligible value.
* The similar litters of each parameter refers to non-significant difference p > 0.05.

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

No.	Treatments	pH	Salinity dS/m	Moisture content %	Potassium Mg/gm	Bicarbonates meq/kg
1	Unpolluted soil (control)	7.83 ±0.05 **a**	834.00 ±29.1 **a**	19.96 ±0.25 **b**	6.96 ±0.41 **a**	5.66 ±0.35 **a**
2	Polluted soil	7.93 ±0.05 **a**	910.33 ±28.02 **c**	13.05 ±0.21 **ab**	8.36 ±0.4 **c**	7.26 ±0.15 **c**
3	Polluted soil + bacteria	7.83 ±0.05 **a**	852.00 ±31.95 **ab**	13.88 ±0.8 **ab**	7.13 ±0.25 **ab**	6.40 ±0.2 **b**
4	Polluted soil + fungi	7.86 ±0.05 **a**	890.33 ±20.79 **bc**	13.05 ±0.32 **ab**	7.86 ±0.56 **bc**	6.83 ±0.15 **d**
5	Polluted soil + bacteria +fungi	7.83 ±0.05 **a**	844.67 ±14.18 **ab**	14.12 ±0.97 **a**	7.13 ±0.35 **ab**	5.86 ±0.15 **a**

* Each value represents mean ± standard deviation.
* Nill = negligible value.
*The similar litters of each parameter refers to non-significant difference p > 0.05.

Pollution with hydrocarbons, has a negative effects on agricultural soils as a result of changed chemical and physical properties (15). Soil texture is important to measure; the amount of clay which present in the soil can affect the soil nature, and therefore microbial survival and activity, also pH increment after pollution attributed to bacterial bioremediation of crude oil within the anaerobic conditions in the polluted soil, and reflected the tendency of crude oil spills to elevate soil pH, the released CO_2 caused the elevation of the alkalinity in the treated soil (16). On the other hand the petroleum may possess some direct impacts in lowering pH value, because the microbial actions contributed to change pH by producing organic acids (17). Soil moisture content decreased with hydrocarbons pollution (as in results of all tables for all treatments in comparison with unpolluted soil) and can affect the biodegradation rate due to its influence on hydrocarbons bioavailability, diffusion processes, transfer of produced gases, oxygen availability in the soil, and soil toxicity level (18).
Results revealed that soil was poor of nutrients, as a reason of alkaline pH, alkaline soils demonstrate deficiencies in many micronutrients (19).
Bicarbonates level in soil effecting on pH value, high bicarbonates value keeping pH at alkaline level, also effecting on the uptake of nutrients by plants from soil and metabolism rate (20).

Total bacterial and fungal count:

Results indicated that total bacterial count of crude oil polluted soil and the polluted treated with fungi only decreased significantly, in comparison with total bacterial count of unpolluted soil (control), while the total count of polluted soil treated with bacteria and with the combination of bacteria and fungi increased significantly (Figure 1). The results of total fungal count of crude oil polluted soil was decreased significantly in comparison with total fungal count of unpolluted soil (control), the significant lowest value was for the treatment with bacteria. While the total count increased significantly when treated with fungi (Figure 2).

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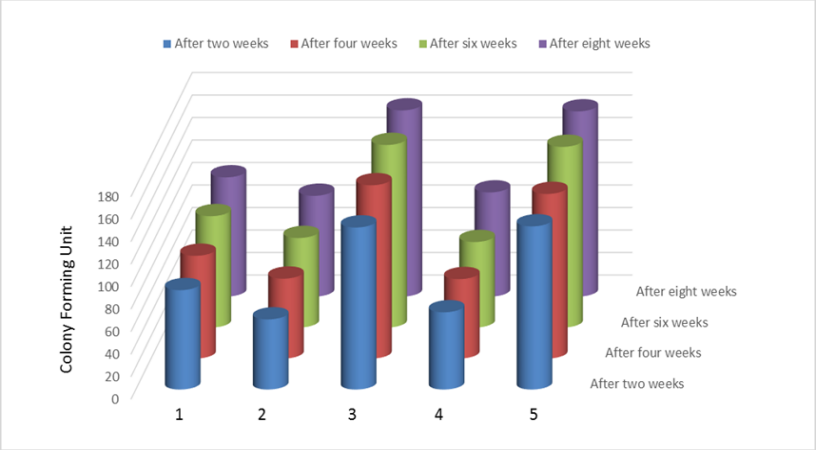

Figure 1: Total bacterial count x 10^9 CFU/gm of the treatments:

(1. unpolluted soil 2. untreated polluted soil 3. polluted soil treated with *P. aeruginosa* 4. polluted soil treated with *P. expansum* 5. polluted soil treated with *P. aeruginosa* and *P. expansum*) during two months.

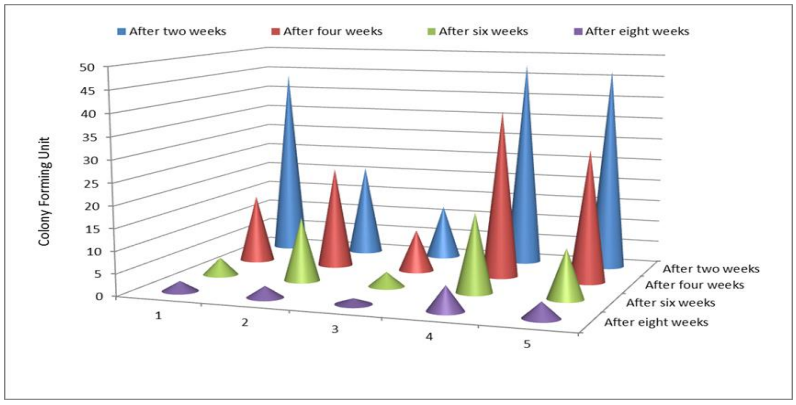

Figure 2 (reversed): Total fungal count x 10^9 CFU/gm of the treatments:

(1. unpolluted soil 2. untreated polluted soil 3. polluted soil treated with *P. aeruginosa* 4. polluted soil treated with *P. expansum* 5. polluted soil treated with *P. aeruginosa* and *P. expansum*) during two months.

Total count of untreated polluted soil was decreased in comparison with unpolluted soil; some microbes will be influenced by reduced permeability of their cell membranes, by blocking or lowering the ability to uptake of nutrients which finally results in starvation and death, also by the direct exposure to the toxic and growth inhibiting chemicals [21]. The total count of bacteria is influenced by soil physical and chemical properties and by hydrocarbon's type. Also soil properties play an important role in controlling the effects of hydrocarbons pollutants on the microbial function and biodiversity. Clay protects soil microbes from the harmful effects of hydrocarbons which results in the decrease total microbial count [22]. High clay texture will decrease the hydrocarbons bioavailability by sorption therefore count will increase in comparison with sandy texture [22], the studied soil texture has clay percentage about 28%. Another important soil parameter is pH which can affect soil microbe's biodiversity, decreased pH values are related with high heterotrophic bacteria CFU enumeration (23), in compare with the high value of current soil pH, it is slightly alkaline pH ≥ 7.7.

Total CFU count increased through time gradually, this reflected the increment of temperature and moisture content. Temperature influence biochemical reactions rates, several reactions doubled for each 10 °C elevation of temperature, also

bioavailability of water as moisture is essential for microbial growth [24]. Treatments with *P. aeruginosa* (bioaugmentation) indicated a higher total CFU counts than unpolluted soil as a because of the addition of inoculum and success of this bacteria to live in hydrocarbons polluted soil. Native or indigenous microbes found in few quantities which cannot reduce polluted region; they don't have the capacity to degrade certain pollutants or they may be in an inactive metabolic case in their environments, for that bioaugmentation is more favor than biostimulation [25]. Total fungal CFU counts of polluted soil was significantly decreased with the time even when treated with *P. expansum*, the lower significant value was of the treatment with *P. aeruginosa*. The results are in line with the study of [26] who studied the ability of two fungal species *Aspergillus* and *Penicillium* dominant at acidic and alkaline soil pH; he found that the lower *Aspergillus* populations whereas at alkaline conditions of (pH = 8 and 8.5) *Aspergillus* were predominant while *Penicillium* was not detected. On the other hand, fungal populations were recorded as the greatest value at acidic soil pH =5.5. While the soil pH of this study was alkaline which is not suitable for many fungal species including *Penicillium.* Total CFU fungal count decreased with the increment of temperature. Temperature and moisture content, are the most important environmental parameters affecting growth of microorganism and activity in the soils, fungi are more adapted to low soil moisture content than bacteria [27]. [28] recorded that microbial growth rates had optimum temperatures about (25–30) C, while lower values at elevated temperatures were recorded, fungi were more effected than bacteria, resulting in an increased growth ratio of bacterial than fungal at higher temperatures which indicating that fungi were more adapted to low-temperature conditions than bacteria. The antagonism relationship between *Pseudomonas* bacteria and *P. expansum* was another important cause for low CFU fungal counts, this relationship was recorded by [29] when added *Pseudomonas* as a biocontrol for *Penicillium expansum*, inhibition fungal rate of fungal growth reached 78.5%.

Polycyclic aromatic hydrocarbons in uncultivated polluted soil:

Concentrations of twelve of polycyclic aromatic hydrocarbons were measured each two weeks through two months, results revealed a complete removal of Benz (a) anthracene, Chrysene, Benzo (b) fluoranthene and Dibenz (ah) anthracene after two weeks of the experiments. Graduated decrease in concentrations was observed for Naphthalene, Tetraphan, Acenaphthylene, Flourene, Phenanthrene, Anthracene, Pyrene and Ovalene when measured each two weeks. Results indicated that PAHs degradation increased with temperature elevation and passage of time, also the inoculation with bacteria and the combination between bacteria and fungi is more effective than inoculation with fungi in comparison with degradation of untreated polluted soil when measured at the same time. Results revealed there is a complete degradation of Tetraphan, Acenaphthylene and Anthracene of polluted soil in addition to those completely treated at the first measurement, while the other compounds concentrations were highly reduced when compared with their concentrations at the beginning as in tables (4), (5), (6) and (7).

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

No.	PAHs	Unpolluted soil	Polluted soi	Polluted soil +bacteria	Polluted soil +fungi	Polluted soil +bacteria +fungi
1	Naphthalene	N.D	**122.02**	**30**	**30**	**19.31**
2	Tetraphan	N.D	**365.24**	**24**	**110.42**	**21.5**
3	Acenaphthylene	N.D	N.D	**21.52**	**N.D**	**23.2**
4	Flourene	N.D	**193.06**	**15**	**20.52**	**10.5**
5	Phenanthrene	N.D	**107.69**	**1.36**	**27.67**	**6.09**
6	Anthracene	N.D	N.D	**4.18**	**5.97**	**3.78**
7	Pyrene	N.D	**4.86**	**1.93**	**4.3**	**1.37**
8	Benz(a)anthracene	N.D	N.D	N.D	N.D	N.D

9	ovalene	N.D	N.D	**10.9**	**51.27**	**15**
10	chrysene	N.D	N.D	N.D	N.D	N.D
11	Benzo (b) fluoranthene	N.D	N.D	N.D	N.D	N.D
12	Dibenz (ah) anthracene	N.D	N.D	N.D	N.D	N.D
13	Total PAHs	N.D	**792.87**	**108.89**	**250.15**	**100.75**

*N.D = Not Detected

Table 5: PAHs concentrations (mg/gm) in crude oil polluted soil after four weeks at 27° c

No.	PAHs	Unpolluted soil	Polluted soil	Polluted soil +bacteria	Polluted soil +fungi	Polluted soil +bacteria +fungi
1	Naphthalene	N.D	**105.5**	**16.95**	**22.16**	**16**
2	Tetraphan	N.D	**46.9**	**8.27**	**13.16**	**7.93**
3	Acenaphthylene	N.D	N.D	**11.37**	**17.76**	N.D
4	Flourene	N.D	**26.22**	**5.31**	**9.01**	**8.02**
5	Phenanthrene	N.D	**15.36**	N.D	**5.88**	**7.21**
6	Anthracene	N.D	N.D	**1.08**	**2.39**	N.D
7	Pyrene	N.D	N.D	N.D	**0.69**	**1.08**
8	Benz (a) anthracene	N.D	N.D	N.D	N.D	N.D
9	ovalene	N.D	N.D	**8.17**	**4.1**	**11.64**
10	chrysene	N.D	N.D	N.D	N.D	N.D
11	Benzo (b) fluoranthene	N.D	N.D	N.D	N.D	N.D
12	Dibenz (ah) anthracene	N.D	N.D	N.D	N.D	N.D
13	Total PAHs	N.D	**211.98**	**51.15**	**75.15**	**51.88**

*N.D = Not Detected

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

No.	PAHs	Unpolluted soil	Polluted soil	Polluted soil +bacteria	Polluted soil +fungi	Polluted soil +bacteria +fungi
1	Naphthalene	N.D	**21.09**	**7**	**18.88**	N.D
2	Tetraphan	N.D	**5.94**	**6.5**	**9.8**	N.D
3	Acenaphthylene	N.D	N.D	N.D	**7**	N.D
4	Flourene	N.D	N.D	**3.4**	**5.37**	N.D
5	Phenanthrene	N.D	**0.96**	N.D	**3.14**	**4.8**
6	Anthracene	N.D	N.D	N.D	**0.78**	N.D
7	Pyrene	N.D	**0.13**	**0.5**	N.D	**0.9**
8	Benz (a) anthracene	N.D	N.D	N.D	N.D	N.D
9	ovalene	N.D	N.D	N.D	**2.9**	**10.4**
10	chrysene	N.D	N.D	N.D	N.D	N.D
11	Benzo (b) fluoranthene	N.D	N.D	N.D	N.D	N.D
12	Dibenz (ah) anthracene	N.D	N.D	N.D	N.D	N.D
13	Total PAHs	N.D	**28.12**	**17.4**	**47.87**	**16.1**

***N.D = Not Detected**

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

No.	PAHs	Unpolluted soil	Polluted soil	Polluted soil +bacteria	Polluted soil +fungi	Polluted soil +bacteria +fungi
1	Naphthalene	N.D	**2.8**	N.D	N.D	N.D
2	Tetraphan	N.D	N.D	N.D	N.D	N.D
3	Acenaphthylene	N.D	N.D	N.D	N.D	N.D
4	Flourene	N.D	N.D	**3.39**	**3**	N.D
5	Phenanthrene	N.D	**1.46**	N.D	N.D	**0.5**
6	Anthracene	N.D	N.D	N.D	N.D	N.D
7	Pyrene	N.D	N.D	N.D	N.D	**0.8**
8	Benz (a)	N.D	N.D	N.D	N.D	N.D

	anthracene					
9	ovalene	N.D	N.D	N.D	N.D	**1.2**
10	chrysene	N.D	N.D	N.D	N.D	N.D
11	Benzo (b) fluoranthene	N.D	N.D	N.D	N.D	N.D
12	Dibenz (ah) anthracene	N.D	N.D	N.D	N.D	N.D
13	Total PAHs	N.D	**4.26**	**3.39**	**3**	**2.5**

*N.D = Not Detected

PAHs considers as a highly hazardous pollutants because they are mutagenic and carcinogenic, their availability in foods threats the human health (30). The Environment Protection Agency named the following PAHs: benz (a) anthracene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (k) fluoranthene, chrysene, dibenz (ah) anthracene, and indeno (1,2,3-cd) pyrene as probable human carcinogens [31]. This study included four of the seven carcinogenic compounds. Results indicated that there is a complete degradation of Benz (a) anthracene, Chrysene, Benzo (b) fluoranthene and Dibenz (ah) anthracene after two weeks of pollution. The elevated concentrations were for Naphthalene, Tetraphan, Phenanathrene and Ovalene. Although the low molecular weight compounds were with high concentrations but the results showed that these compounds were greatly reduced after eight months from the beginning of the experiments in comparison with high molecular weight compounds because lower molecular weight PAHs are more volatile than higher molecular weight PAHs [32]. The results of this study revealed that there is a slow rate degradation of PAHs from untreated polluted soil in comparison with treated polluted soil. [7] documented that the crude oil will not persist for long period even if the spilled quantity was high, because of the role of volatilization by temperature, photolysis by sunlight and followed by degradation of endogenous soil microorganisms. The results of PAHs photolysis of the study of [33] revealed that pyrene was more resistant while fluoranthene was not resistant, also documented that the photolysis is an effective process to remove PAHs compounds. Treatment of polluted soil with bacteria and the combination of bacteria and fungi were the most effective than the treatment with fungi in comparison with untreated polluted soil, this is in line with study of (34), he recorded that *Pseudomonas* is the most active petroleum hydrocarbons degrader among other microorganisms. The [35] studied the capacity of endophytic bacteria *Stenotrophomonas* sp. and *Pseudomonas* to breakdown PAHs, the two endophytic bacteria removed more than 90% of phenanthrene during one week. Both bacteria could utilize naphthalene, phenanthrene, fluorene, pyrene, and benzo (a) pyrene as a main sources of carbon, these bacteria decreased PAHs pollution within one week. Also results revealed that bioremediation rate increased with temperature elevation that is because of evaporation is considered as another mechanism that increase the bioremediation rate (36). Results showed that high molecular weight PAHs compounds degraded with low rate when compared with low molecular weight PAHs. [37] investigated the aging of PAHs in their environments, they indicated that the half -life of PAHs compounds increased with increased number of rings and the molecular weight for each compound, assuming that more than three rings compounds will take more time to degrade.

References:

[1] O'Rourke, D and. Connolly, S. Just oil? the distribution of environmental and social impacts of oil production and consumption. Reviews in Advance. 28: 1-31. 2003.

[2] Wang, Y., Xu, Z., Bach, S. J. and McAllister, T. A. Effects of phlorotannins from Ascophyllum nodosum (brown seaweed) on in vitro ruminal digestion of mixed forage or barley grain. Anim. Feed Sci. Technol. 145 (1/4): 375-395. 2008.

[3] Benka-Coker, M .O. and Ekundayo, J. A. Effect of an oil spill on soil physic-chemical properties of a spill site in the Niger Delta area of Nigeria. Environ. Monitor. Assess. 36: 93 -104. 1995.

[4]Johnson, R. B., Onwuegbuzie, A. and Turner, L. Toward a definition of mixed methods research. Journ of Mix Meth Res. 1: 112-133. 2007.

[5] Iranzo, M., Sainz-Padro, I., Boluda, R., Sanchez, J. and Mormeneo, S. The Use of Microorganisms in Environmental Engineering, Annals of Microbiology. 51: 135-143. 2001

[6] Boldu-Prenafeta, F. X., Ballerstedt, H., Gerritse, J. and Grotenhuis, J. T. C. Bioremediation of BTEX hydrocarbons: effect of soil inoculation with the toluene-growing fungi Cladophialopora sp. Strain T1. Biodegradation. 15 (1): 59–65. 2004

[7] Akpoveta, O.V.; Egharevba, F. and Medjor, O.W. A pilot study on the biodegradation of hydrocarbon and its kinetics on Kerosene simulated soil. International Journal of Environmental Science. 2 (1): 54-67. 2011

[8] Sathishkumar, J., Saravanan, M. M. and Seethalakshmi, I. In-vitro Antimicrobial and Antitumor Activities of Stevia Rebaudiana (Asteraceae) Leaf Extracts. Trop. J. Pharm. Res. 7:1143-1149. 2008

[9]Aburas, M. M. Enhancing Bioremediation of Saudi Desert Soils Polluted with Hydrocarbons. Doctoral thesis submitted to University of Surrey. 2010.

[10] Bashour, I. I. and Sayegh, A. H. Methods of Analysis for Soils of Arid and Semi-Arid Regions. 1st Edn., FAO, USA. p. 119. 2007

[11] Ryan J.; Estefan, J. and Rashid, A. Soil and Plant Analysis Laboratory Manual. Jointly published by the International Centre Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria and National Agric. Res. Centre (NARC), Islamabad. 2001.

[12] Nandhini, B. and Josephine, M. R. A. Study on bacterial and fungal diversity in potted soil Int.J.Curr.Microbiol.App.Sci, Vol.2 No.2, pp. 1-5. 2013

[13] Sarhan, A. R. T. Practical Mycology. First Edition. 2012.

[14] Arbabi, M.; Nasseri, S.; Mesdaghinia, A. R.; Rezaie, S.; Naddafi, K.; Omrani, G. H. and Yunesian, M. Survey on physical, chemical and microbiological characteristics of PAH contaminated soils in Iran. Iranian J Env Health Sci Eng, Vol.1, No. 1, pp. 26-33.2004.

[15] Agbogidi, O. M., Eruotor, P. G., and Akparabi, S. O. Effects of Time of Application of Crude Oil to Soil on the Growth of Maize (Zea mays L.). Res Jour of Envir Toxicol. 1(3): 116- 123. 2007.

[16] Essien, O. E. and John, I. A. Impact of crude-oil spillage pollution and chemical remediation on agricultural soil properties and crop growth. J. Appl. Sci. Environ. Management. 14 (4): 147 – 154. 2010

[17] Manahan, S. E. Environmental Chemistry. CRC Press, Inc. Florida. pp. 811. 1994

[18] Yang, S. Z., Jin, H. J., Wei, Z., He, R. X., Ji, Y. J., Li, X. M. and Yu, S. P. Bioremediation of oil spills in cold environments: A review. Pedosphere. 19 (3):371-381. 2009.

[19] Hopkins, B. and Ellsworth, J. Phosphorus availability with alkaline/calcareous soil. Western Nutrient Management Conference. Salt Lake City, UK. 6: 88-93. 2005

[20] Jarrahi, N., Ardalan, M. M. and Amir, N. A. Effect of bicarbonate of irrigation water on absorption of some of micro elements and leaf chlorophyll of some Citrus rootstocks in hydroponic culture. IJACS. 6(7): 389-395. 2013

[21] Pezeshki, S. R., Hester, M. W., Lin, Q. and Nyman, J. A. The effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: a review. Environmental Pollution. 108: 129-139. 2000

[22] Labud, V., Garcia, C. and Hernandez, T. Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. Chemosphere. 66: 1863-1871. 2007

[23] Killham, K. Soil Ecology. (4th ed.) Cambridge University Press, Cambridge, UK. 2006

[24]Thapa S. B., Evaluation of twenty bermudagrass cultivars for their drought resistance. Master thesis. Oklahoma State University. Nepal. 2011

[25] Annibale, A., Sermanni, G. G., Federici, F., and Petruccioli, M. Olive-mill wastewaters: a promising substrate for microbial lipase production. Bioresour. Technol. 97: 1828 – 1833. 2006

[26] Pawar, R. M. The Effect of Soil pH on Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHS). J Bioremed Biodeg 6:291. 2015

[27] Paul, E. A. and Clark, F. E. Soil Microbiology, Ecology, and Biochemistry. USA: Academic Press. 1996.

[28] Pietikainen, M., Valiheikki, A., Oravisjarvi, K., Kolli, T., Huuhtanen, M., Niemi, S., Virtanen, S., Karhu, T. and Keiski, R.L. Particle and NOx emissions of a non-road diesel engine with an SCR unit: The effect of fuel, Renewable Energy. 77: 377-385. (2015

[29] Etebarian, H. R., Sholberg, P. L., Eastwell, K. C. and Sayler, R. J. Biological control of apple blue mold with Pseudomonas fluorescens Can. J. Microbiol. 51: 591–598. 2005

[30] Jánská, M., Hajslová, J., Tomaniová, M., Kocourek, V. and Vávrová M. Polycyclic aromatic hydrocarbons in fruits and vegetables grown in the Czech republic. Bull Environ Contam Toxicol. 77: 492-499. 2006

[31] United States Environmental Protection Agency (USEPA). Polycyclic aromatic hydrocarbons (PAHs) -EPA fact sheet. Washington (DC): National Center for Environmental Assessment, Office of Research and Development. 2008

[32] CCME (Canadian Council of Ministers of the Environment). Canadian Soil Quality Guidelines for Carcinogenic and Other Polycyclic Aromatic Hydrocarbons (Environmental and Human Health Effects). Scientific Criteria Document (revised). 2010

[33] EL –Saeid, M. H., Al-Turki, A. M., Nadeem M. E. A., Hassanin A. S. and Al-Wabe M. I. Photolysis degradation of polyaromatic hydrocarbons (PAHs) on surface sandy soil Environ Sci Pollut Res. ISSN 0944-1344I. 2015

[34] Saadoun I. Isolation and characterization of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel fuel, Journal of Basic Microbiology. 42:420-428. 2002.

[35] Zhu, X., Ni, X., Waigi, M. G., Liu, J., Sun, K. and Gao, Y. Biodegradation of Mixed PAHs by PAH-Degrading Endophytic Bacteria Int. J. Environ. Res. Public Health. 13, 805; doi:10.3390/ijerph13080805. 2016

[36] McCain, W. D. The properties of petroleum fluids. PennWell Books, Tulsa. 1990

[37] Kim, D., Kumfer, B. M., Anastasio, C., Kennedy, I. M. and Young, T. M. Environmental ageing of polycyclic aromatic hydrocarbons on soot and its effect on source identification. Chemosphere. 76: 1075-1081. 2009