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Quantification of Polycyclic Aromatic Hydrocarbons (PAHs) Loss in Crude Oil Contaminated Soil Using Phytoremediation Technology

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are group of chemicals composed of two or more fused aromatic rings that are formed from the incomplete combustion or high-temperature pyrolysis of coal, crude oil, burning for agricultural farming…etc., they diffuse in the environment and release to air, soil, water and food. Some PAHs have shown to have toxicological, carcinogenic and mutagenic effects on animals and humans. Phytoremediation of crude oil contaminated soils presents promising technology for environmental cleanup using in situ treatment, especially in the developing countries. The objective of this work is bio stimulation to enhance the efficiency of phytoremediation technology for removal of PAHs from petroleum hydrocarbon contaminated soil by using humic acid (HA) with a legume plants (Faba bean or Alfalfa) and quantify the dissipation amount and dissipation ratio of PAHs as consequence to bioremediation processes in these cases (individually or combined). In the present study, soil and plant samples were collected from three different farms adjacent to the refinery station at Kafr Al-Elow village, Helwan, Cairo governorate, Egypt, which were exposed to polycyclic aromatic hydrocarbons (PAHs) derived from crude oil, caused by leaking storage tanks or spillage during their transport since a few years ago. These farms were treated with traditional methods (irrigating, drying, tilling and planting... etc.) by the farmers. Results showed high concentration of total heavy metals, i.e. Cu, Zn, Fe, Cd, Ni and Pb and total (PAHs) existing in surface soil (0-20 cm), these values vary within the studied farms. It was noticed that the crude oil was accompanied by high concentration of either organic pollutants (PAHs) and inorganic pollutants (heavy metals and trace elements), however, it decreased by depth due to their low mobility. The losses in total PAHs was recorded in case of soil planted with Alfalfa > Wheat > Sorghum respectively, thus the legume plants could be considered unsuitable candidates for phytoremediation of soils contaminated with PAHs pollutants. Also, the results indicate that the translocation of PAHs from root to shoot was considerably restricted. Pots experiment was conducted on heavily contaminated soil to quantify the losses of total PAHs according to different bioremediation processes. The results indicate that dissipation ratio of total PAHs increased in rhizospheric soil by time. It was (34.1, 48.6 and64.6%) for 30, 60 and 90 days respectively after sowing the soil by Alfalfa combined with humic acid addition. But these increases in dissipation ratio were little in case of planting Faba bean combined with humic acid addition as phytoremediation technology, as it reached (25.7, 39.8 and 44.8%) at the same period respectively. On the other hand, the dissipation ratio in case of humic acid addition alone to soil was less than above treatments, as it reached (7.8, 24.6 and 41.3%), respectively.

Key words: Contaminated soil, Crude oil, Phytoremediation, Humic acid and Legume plants.

Introduction

Soil contamination by petroleum hydrocarbons caused by leaking storage tanks, spillage during their transport, abandoned petrochemical manufacturing facilities, and industrial facilities has attracted considerable public attention over the past decades (Peng *et al*., 2009). There are many industrial areas which produce large amount of polycyclic aromatic hydrocarbons (PAHs) in Egypt, which represent severe hazards effects on the environment. (Farahat and El gendy, 2008 and Abd-El Salam *et al*., 2009). Many PAHs are reasonably expected to be carcinogenic and suspected to cause birth defects (ATSDR 2014).They are diverse class of persistent hydrophobic organic compounds. Some PAHs are considered potential carcinogens, mutagens and teratogens by the Environmental Protection Agency (USEPA 2011) and the Agency for Toxic Substances and Disease Registry (ATSDR 2010) and are listed in the National Waste Minimization Program of the U.S Environmental Protection Agency and the European Union as priority pollutants. According to the criteria established by Maliszewska-Kordybach, (1996), four classes

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of soil contamination were identified based on a total of 16 PAHs: non contaminated soil (<200 ppm), weakly contaminated soil (200 - 600 ppm) contaminated soil (600 - 1000 ppm) and heavily contaminated soil (>1000 ppm).

Research on the origins and geochemistry of PAHs revealed that anthropogenic PAHs can be categorized as pyrolytic and petrogenic. Pyrolytic PAHs result from incomplete fuel combustion, wood and coal burning, car emissions, tobacco related activities and meat grilling (Farhadian *et al*. 2010). This type of PAHs is also found in coal tar, creosote, roofing tar and parking lot seal coats (ATSDR 2010). Petrogenic PAHs are mainly derived from crude oil, unburned fuel and refinery products (Elias *et al*. 2007). Large concentrations of PAHs are expected to be present in urban areas and in areas where bush burning for agricultural farming is commonly practiced as well as in petroleum exploration and refining operations (Fetzer, 2000).PAHs in soils are usually found in mixtures, and present for many years, its sequestration are the result of adsorption to soil particles and organic matter (Bogen and Sullivan 2003). With time, PAHs get trapped in soil micropores, which increases persistence and limit biodegradability, leachability, and volatility of PAHs (Wick *et al*. 2011).

Several remediation techniques which are efficient and cost effective in removing PAHs from the environment, such as the conventional clean-up methods which are not only environmental friendly; but also present a novel approach in reducing the ability of PAHs to cause prospective risk to humans and the ecosystem.(Ukiwe *et al*.,2013).The commonly employed biological methods for PAHs remediation in soil are land farming, composting, bio augmentation, bio stimulation, phytoremediation etc. whereas the chemical methods include photooxidation, ozone treatment, Fenton processes etc. (Nilanjana and Develina, 2015). If soils are contaminated by both heavy metals and toxic organic contaminants, it is very difficult to remedy using bioremediation due to the adverse effects of heavy metals on organic matter degradation rate (Mitsch and Jørgensen, 2004), and seriously affected by the potential toxicity of heavy metals on microorganisms (Gadd and Griffiths, 1978). Bioremediation of contaminated soils with PAHs can be simple, cost-effective and ex situ or in situ. More research is needed to fully understand mechanisms of bacterial degradation of PAHs (Gloria Patricia Johnston, 2014).

Recent studies have shown the suitability of phytoremediation as a feasible technology for decontaminating soils polluted by PAHs (Soleimani, *et al*., 2010). Phytoremediation is an emerging on-site green remediation strategy that uses plants to reclaim contaminated soil containing toxic pollutants mainly through increasing microbial activity in the rhizosphere by breaking down the organic compounds in contaminated soils by metabolic processes (Dzantor and Beauchamp, 2002) and is an inexpensive technology widely used (Lin and Mendelssohn, 2009). Plants can enhance microbial degradation by providing oxygen in the root area along root channels and loosened soil aggregates. Molecular oxygen is required for substrate oxidation which is the initial step in the degradation of most hydrocarbons (Yeung *et al*., 1997). The success of phytoremediation depends on the amount of contamination and the type of plants selected (Conesa and Shulin, 2010) and on microbial degradation, both aerobic and anaerobic, (Gloria Patricia Johnston, 2014). In general low molecular weight PAHs can be more effectively removed by phytoremediation. Disadvantages of phytoremediation include low uptake by plants, uneven distribution of roots in soils, soil heterogeneity, and contaminant heterogeneity (Conesa and Shulin, 2010).

Bio stimulation is another bioremediation technique which consists of increasing the degrading capacity of the indigenous community by adding nutrients to avoid metabolic limitations (Vinas, *et al*., 2005). Plant growth is influenced directly and indirectly by humic substances (HS). Direct effects include those changes in plant metabolism that occur following the uptake of humic and fulvic acid. Indirect effects influence the soils water holding capacity, soil structure, release of plant nutrients from soil minerals, increased availability of micronutrients and improved soil fertility. Humic substances have been directly correlated with enhanced uptake of macronutrients such as N, P, K and S, and micronutrients such as Fe, Zn, Cu and Mn. (Youssef, and Abd El-Rheem, 2015). Where, Humic acid, one of the most important components of HS, help break up clay and compacted soils, assist in transferring micronutrients from soil to plants, enhance water retention, increase seed germination rates, and stimulate the development of microflora populations in soils. Humic acid also slow down water evaporation from soils as it is the most active components of soil and compost organic matter. HA have been shown to stimulate plant growth and consequently yield by acting on mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities (Chen, *et al*., 2004).The type and extent of interaction between organic pollutant and HS may change with time and eventually may result in complete immobilization and incorporation of the specific substance in the humic polymer (Bollag, *et al*., 1983 and Stott, *et al*., 1983). Humic acid (HA) has been found to affect the solubility, mineralization, and bound residue formation of polycyclic aromatic hydrocarbons (PAHs). However, most of the studies on the interaction between HA and PAH concentrated on one or two of the three phases. (Yanna *et al*., 2008). Humic substances (HS), naturally occurring surfactants, begin to be recognized as a possible aid in soil bioremediation techniques. The bioavailability of Polycyclic Aromatic Hydrocarbons (PAH) appeared to be increased by addition of exogenous humic substances to contaminated soil. Furthermore, the surfactant activity of HS was found to reduce sorption of organic contaminants on spiked soils, thereby enabling desorption-remediation of PAH and heavy metals (Halim, *et al*., 2003). The objective of this work is quantification of PAHs loss in crude oil contaminated soil following some bioremediation process consequently by addition HA, which improve or biostimulate the role of legume plants (Faba bean or Alfalfa) in phytoremediation technology.

Materials and Methods

To achieve the main objective of this work, the following studies were carried,

Field work:

Soil and plant samples were collected from three different farms adjacent to the refinery station at Kafr Al-Elow village. Helwan, Cairo governorate, Egypt, which were exposed to Polycyclic aromatic hydrocarbons(PAHs) derived from crude oil, caused by leaking storage tanks or spillage during their transport since few years ago. These farms were treated with traditional methods (irrigating, drying and tilling ...etc.) by the farmers and planted with different species of plants, i.e. Alfalfa, (*Medicago Sativa, L.*); Sorghum, (*Sorghum Vulgare*) and wheat *(Triticum aestivum)*.Three soil samples were collected from each farm at two depths (0-25 cm, 25-40 cm) to determine total heavy metals and PAHsresidue in soil layers and to determine the availability of heavy metals at the end of plant age approximately. Plant samples were taken thenseparated to shoot and root and prepared to determine its contents of PAHs and heavy metals.

Pot experiment:

To quantify the bioremediation process in the contaminated soil with PAHs and heavy metals, one of the three studied farms were selected for conducting a pot experiment. This soil is clay loam with a pH of 7.1, EC 1.52 $dS.m^{-1}$, organic matter, 2.8%.A total of 15 pots were prepared in a randomized block design, each pot was filled by 3kg from this contaminated soil containing 2625 mg kg^{-1} of total PAHs for testing the different treatments with three replicates as follows:

(T_1) Contaminated soil only (control);

(T_2) Contaminated soil amended with HA (1000 mg/kg) + and microbe-inhibited (0.5% $HgCl_2$ was used to inhibit the microbial activity);

(T_3) Contaminated soil amended with HA (1000 mg/kg) without microbe-inhibited (control+ HA);

(T_4) Contaminated soil amended with HA and planted with Alfalfa plant.

(T_5) Contaminated soil amended with HA and planted with faba bean plant.

During the experimental period (90 days), each pot was watered every day with distilled water to compensate evapotranspiration loss. Soil and plant samples were collected from each treatment after 30, 60 and 90 days from sowing (zero time for experiment) and prepared to analysis.

Soil samples:

The collected soil samples were air-dried and passed through a 2-mm sieve prior to use. 1.0 g of homogenized soil sample was digested with 12.5 ml of aqua regia (HNO_3: HCl : $HClO_3$ with a ratio 3:1:1), the samples were heated until the color becomes clear, dissolved with several drops of 1% HNO_3, filtered, diluted to a volume of 50 ml with distilled water, and analyzed for the total content of heavy metals using atomic absorption spectrophotometer, described by Jackson (1973). Ten grams of the air-dried soil samples were soxhelet extracted using a mixture of n-hexane and dichloromethane (1:1v/v), all extracted samples were analyzed using HPLC to determine total PAHs (Viguri *et al*., 2002).

Plant samples:

Plants Samples were collected, separated to shoots and roots and washed with distilled water to be free of soil. Each plant portion was dried at 70°C, crushed and wet digested using a mixture of H_2SO_4 +

$HClO_4$ acids to determine heavy metals contents in aliquots of the digested solutions, (Ryan *et al*., 1996). Dry shoot and root samples were ground and extracted (100~200 mg dry weight) for 4 hours using 200 ml chloroform. The extracts were concentrated to 0.5ml and analyzed to determine total PAHs by using HPLC as described by Szolar *et al*., (2002).

Results and Discussion

Total heavy metal and PAHs in soil and plant in the contaminated farms:

Although the different farms adjacent to the refinery station are exposed to the same sources of petroleum hydrocarbon pollution, the data in table (1) shows different values of heavy metal and total PAHs in soil depending on the species of plant cultivated. The lowest concentration of total PAHs was recorded in the rhizospheric soil planted with Alfalfa (1400 $mg.kg^{-1}$) followed by Wheat (2250 $mg.kg^{-1}$) and Sorghum (2625 $mg.kg^{-1}$), respectively. Thus the legume plants were suitable candidates for phytoremediation of soils contaminated with PAHs pollutants. This may be due to the ability of legume roots to fix atmospheric nitrogen and can exude certain enzymes to degrade or transform PAHs pollutants (Liu *et al*, 2004). Also it was noticed that low values of total heavy metals content in addition to total PAHs existin the deepsoil layers. Thismay be due to the little mobility of the petroleum hydrocarbon pollution in the soil, which exists in complex phase that are of limited mobility and low in water solubility, in addition to its adsorption on the soil colloids, precipitation and interaction with organic ligands (McBride, 1989).The concentration of heavy metalsin plants roots was higher than in their shoots, this phenomena was very clear for PAHs. Generally, these results indicate that the transfer of PAHs from roots to shoots are considerably restricted which may be due to its enormous molecular weight and its lower water solubility.

Table 1: Heavy metal contents, total PAHs in both the contaminated soil and plants

Studied Parameters		Heavy metals contents (mg kg^{-1})							Total PAH_s (mg kg^{-1})
		Cu	Zn	Fe	Mn	Cd	Ni	pb	
Total in soil (1)	0 - 20 Cm	45.0	148.0	1620.0	325.0	2.2	14.8	41.0	2625.0
	20 - 40 Cm	25.0	41.0	800.0	150.0	1.2	9.5	22.0	1800.0
Available in rhizospheric soil (0 - 20 Cm)		5.0	17.0	78.0	35.0	0.5	1.5	4.5	.
content in Sorghum plant	Shoot	33.0	81.0	245.0	111.0	0.4	2.7	9.3	2.8
	Root	38.0	112.0	298.0	187.0	0.7	4.1	12.4	375.0
Total in soil (2)	0 - 20 Cm	41.0	144.0	1570.0	320.0	2.0	14.1	40.0	2250.0
	20 - 40 Cm	25.0	40.0	800.0	145.0	1.0	9.5	20.0	1090.0
Available in rhizospheric soil (0 - 20 Cm)		4.5	16.0	75.0	36.0	0.5	1.5	4.5	.
content in wheat plant	Shoot	32.0	88.0	221.0	119.0	0.3	2.9	9.8	3.4
	Root	40.0	116.0	277.0	173.0	0.6	4.3	12.9	381.0
Total in soil (3)	0 - 20 Cm	38.0	141.0	1535.0	318.0	1.9	12.8	39.0	1400.0
	20 - 40 Cm	25.0	40.0	780.0	150.0	1.2	8.5	18.0	680.0
Available in rhizospheric soil (0 - 20 Cm)		4.0	15.0	73.0	32.0	0.5	1.5	4.0	.
content in Alfalfa plant	Shoot	36.0	93.0	235.0	121.0	0.4	3.1	10.2	5.1
	Root	44.0	123.0	341.0	191.0	0.8	4.4	17.9	492.0

Impact of applied treatments on total heavy metals and PAHs (pot. exp.):

The results shows in Table (2) indicate that the concentrations of heavy metals and total PAHs decreased generally with the time in all treatments. These results are in agreement with (Fabio, *et al*., 2004) who reported that using Humic Substance (HS) as enhancing agent of pollutant bioavailability appears promising for bioremediation of PAHs contaminated soils. On the other hand, loss in total PAHs increased by increasing the prediction bioremediation process with addition of HA to soil planted compared to its addition to unplanted soil (T_3). HA may improve plant growth and mineral nutrition (Atiyeh *et al*. 2002),making phytoextraction a viable option in such hostile conditions, the degradation of organic contaminants by microorganisms could be feasible. HA can increase the degradation potential of organic contaminants by providing favorable conditions for rhizosphere micro organisms. Thus, the planted soil with Alfalfa or faba bean in presence of HA (T_4 and T_5, respectively) were able to reduce total PAHs in rhizospheric soil from 2625 at 0 time to 1730, 1350 and 930 mg. kg^{-1} soil and to 1950, 1580 and 1450 mg. kg^{-1} soil at 30, 60 and 90 days for Alfalfa and faba bean respectively. In contrast, PAHs dissipation values were relatively low in case of HA addition to unplanted pots (T_3) compared the planted ones which may be due to the leaching absence and the role of plant roots in pot experiments. These results show the positive role of HA- rhizosphere legume plant interaction (T_4 and T_5) as it enhanced the biodegradation and dissipation of total PAHs which is different from plant to other. It was noticed that diminishing the total PAHs is of lower rate in cases of faba bean compared to Alfalfa, which may be due to differences in the nature and composition of root exudates that reflects the biodegradation potential of the microbial community in rhizosphere (Corgie *et al*., 2004). More research on (HA-rhizosphereic plant) is needed to fully understand mechanisms of degradation and bioremediation of PAHs under petroleum contaminated sites.

Table 2: Effect of treatments on available heavy metals and total PAHs in rhizospheric soil contaminated with the time.

Studied Parameters		Available heavy metals in rhizospheric soil (mg kg^{-1})							Total PAHs (mg kg^{-1})
Time	Treatments	Cu	Zn	Fe	Mn	Cd	Ni	pb	
30 days	T1	5.0	17.0	78.0	35.0	0.5	1.5	4.5	2625
	T2	4.5	16.0	75.0	35.0	0.5	1.5	4.5	2560
	T3	3.5	15.5	57.0	22.5	0.5	1.5	3.5	2420
	T4	2.5	9.5	31.0	16.5	0.4	0.5	2.5	1730
	T5	2.5	9.0	33.0	17.0	0.4	1.0	2.5	1950
60 days	T1	4.0	13.5	75.0	32.0	0.5	1.5	4.0	2625
	T2	4.0	12.5	60.0	30.0	0.5	1.5	3.5	2430
	T3	2.5	11.0	45.0	15.0	0.5	1.5	3.0	1980
	T4	2.0	7.0	23.0	10.0	0.5	0.5	2.0	1350
	T5	2.0	7.0	27.0	12.0	0.5	1.0	2.0	1580
90 days	T1	3.5	13.0	75.0	30.0	0.5	1.5	4.0	2600
	T2	3.0	12.5	55.0	25.0	0.5	1.5	3.5	2250
	T3	2.0	9.0	35.0	10.0	0.0	1.0	2.0	1540
	T4	1.0	5.0	15.0	4.5	0.0	0.5	1.0	930
	T5	1.5	5.5	20.0	5.0	0.0	0.5	1.5	1450

Quantification of dissipations amount and ratio of PAHs:

The quantification of the dissipations amounts and dissipations ratio of PAHs (Σ of 16 US EPA priority PAH individual)in polluted soil by time were calculated and presented in Table (3).The rhizospheric soil for Alfalfa and faba bean plant (T_4 and T_5)showed maximum dissipations ratio in total PAHs after 90 days by 64.6% and 44.8% respectively. In contrast, 41.3 % dissipations ratio for the non-rhizospheric soil (T_3) after the same time compared the control. On the other hand, this dissipation ratio was reduced to 14.3% using HA with microbe-inhibited (T_2) after 90 days compared to the other treatments, this may be due to the absence of all or most biological process in this condition (microbe-inhibited), in addition to absence of leaching system in pot experiment. The role of HA declined at its addition to contaminated soil in absence of abiotic system (microorganisms and plant roots) although it is able to increase the solubility of PAHs in soil. Data calculated in table (3) showed that the highest loss in concentration of total PAHs was recorded in the rhizospheric soil which was planted with Alfalfa (T_4) then faba bean (T_5) followed by non rhizospheric soil (T_3) and (T_2) respectively, thus the legume plants were suitable candidates for phytoremediation of soils contaminated with PAHs particularly Alfalfa plant in this condition.

Table 3: Effect of applied treatments on loss of total PAHs in planted soil contaminated

Treatments	PAHs (mg.kg^{-1}) after 30 days					
	Initial concentration	Rhizospheric soil	Roots	Shoots	Dissipation amount	Dissipation ratio (%)
T_1 (Control)	2625.0	2625			0	0.0
T_2		2560	-	-	65	2.5
T_3		2420	-	-	205	7.8
T_4		1730	128	2.5	895	34.1
T_5		1950	85	2.0	675	25.7
Treatments	PAHs (mg.kg^{-1}) after 60 days					
	Initial concentration	Rhizospheric soil	Roots	Shoots	Dissipation amount	Dissipation ratio (%)
T_1 (Control)	2625.0	2625			0	0.0
T_2		2430	-	-	195	7.4
T_3		1980	-	-	645	24.6
T_4		1350	140	2.5	1275	48.6
T_5		1580	130	2.5	1045	39.8
Treatments	PAHs (mg.kg^{-1}) after 90 days					
	Initial concentration	Rhizospheric soil	Roots	Shoots	Dissipation amount	Dissipation ratio (%)
T_1 (Control)	2625.0	2600			25	1.0
T_2		2250	-	-	375	14.3
T_3		1540	-	-	1085	41.3
T_4		930	160	2.5	1695	64.6
T_5		1450	0.0	0.0	1175	44.8
T_1 (Control= Sc untreated)		T_3 (Sc Treated with HA)		T_4 (treated with HA+ planted by Alfalfa)		
T_2 (Sc treated with HA+Hg)				T_5 (treated with HA+ planted by Faba bean)		

- *Dissipation amount, represent the mean of the three replica.*
- *Dissipation amount T = (Ci - Cr)*
- *Dissipation ratio (% A) = T × 100/Ci*
- *Where Ci was the soil initial concentration (mg kg^{-1}).*
- *Cr was the soil residual concentration (mg kg^{-1}) after 30, 60 and 90 days.*

Generally, results calculated and presented in Table (3) participated in quantifying the dissipation amount and dissipation ratio of total PAHs from the polluted soil as a consequence ofeach treatment.Addition of HA to contaminated planted soil improve remediation in the rhizosphere by encouraging healthy plant growth and thus enhancing microbial activity. Thelaboratory pot experiment present an approach model to quantify the role of each factor alone in bioremediation processor phytoremediation technique to degrade total PAHs in contaminated planted soil. Dissipation of PAHs in planted soil with legume plants included evaporation, a biotic dissipation, biodegradation and plant uptake and accumulation. In contrast, the dissipation of these compounds in unplanted soils was the sum of evaporation, a biotic dissipation and biodegradation. Thus the loss of total PAHs in planted and unplanted soils could be expressed as:

$$D_p = D_e + D_b + D_d + D_{pa} \quad (1)$$

$$D_{unp} = D_e + D_b + D_d^* \quad (2)$$

Where D_p and D_{unp} were the dissipation of PAHs in contaminated planted and unplanted soils (mg kg^{-1}). D_e and D_b denoted the dissipation by evaporation and abiotic dissipation respectively. D_d and D_d^* were the loss by biodegradation in planted and unplanted soils, respectively. D_{pa} denoted the removal of PAHs by plant uptake and accumulation. Thus, the dissipation enhancement (D_{en}) of PAHs in planted versus unplanted soils was

$$D_{en} = D_p - D_{unp} = (D_d – D_d^*) + D_{pa} \quad (3)$$

$$D_{pp} = D_d – D_d^* \quad (4)$$

Where D_{pp}denoted the loss of total PAHs by the plant- promoted biodegradation. Obviously, the enhanced dissipation of total PAHs in planted versus unplanted would strongly derive of plant direct uptake and accumulation and promoted biodegradation.

Data calculated and presented in Table (4) showed that dissipation amount or dissipation ratio of PAHs in planted soil (D_p) were of maximum values compared with other treatments, where the plants contribute to the biodegradation of organic pollutants by increasing microbial number (Reilley *et al.*, 1996; Binet *et al.*, 2001), a modification in microbial community in rhizosphere (Joner *et al.*, 2003) and a promotion in microbial activity (Binet *et al.*, 2000; Newman and Charles, 2005). These values increased from 34.1%, 25.7% to 48.6%, 39.8% and 64.6%, 44.8% with increasing the time at 30,60 and 90 days for T_4 and T_5 respectively, compared with the concentration of total PAHs at 0 time (2625 mg.kg^{-1}). The dissipation amount enhancement of PAHs (mg/kg^{-1}) in planted contaminated soil (D_{en}) was calculated by the sum of plant-promoted biodegradation (D_{pp}) and plant accumulated (D_{pa}). The plant-promoted biodegradation of PAHs was of major contribution, and larger than 80.9%, 77.3% and 73.3% of the enhancement of PAHs dissipation in planted contaminated soil with Alfalfa plant at 30,60 and 90 days after sowing.Its contribution was 81.5%, 66.4% and 100.0% in soil planted with faba bean at the same time respectively, while it is expected that plant roots enhance the pools of extractable oxido-reductases enzymes as well as other types of enzymes by root exudation. In addition, the presence of rhizosphere microorganisms increases root exudation by plants (Gramss *et al.*, 1999; Newman and Reynolds, 2005). However, amount of PAHs directly accumulated by the tested plant (D_{pa}) contributed by the remainder ration in the enhancement in dissipation of PAHs. The dissipation ration of PAHs pollutants by native microorganisms (D_m) in planted soil was increased from 5.3% to 17.1% then 27.0% with increasing the time at 30, 60 and 90 days for each Alfalfa or faba bean plant, also The dissipation ration of PAHs in planted soil was enhanced by HA, this dissipation (D_{HA}) had the same trend of (D_m)it was increased from 2.5% to 7.4% and 14.2%, respectively for the same time and plants.

Table 4: Quantification of the role plants in enhancement and promotion of PAHs remediation in planted versus unplanted contaminated soil with initial PAHs concentration of 2625 mg kg^{-1}

****		PAHs (mg.kg^{-1}) after 30 days					
		D_p	D_{en}	D_m	D_{pa}	D_{pp}	D_{HA}
T_4 (treated with HA+ planted by Alfalfa)	Dissipation amount	895.0	690.0	140.0	130.5	559.5	65.0
	Dissipation ratio (%)	34.1	26.3	5.3	5.0	21.3	2.5
T_5 (treated with HA+ planted by Faba bean)	Dissipation amount	675.0	470.0	140.0	87.0	383.0	65.0
	Dissipation ratio (%)	25.7	17.9	5.3	3.3	14.6	2.5
****		PAHs (mg.kg^{-1}) after 60 days					
		D_p	D_{en}	D_m	D_{pa}	D_{pp}	D_{HA}
T_4 (treated with HA+ planted by Alfalfa)	Dissipation amount	1275.0	630.0	450.0	142.5	487.5	195.0
	Dissipation ratio (%)	48.6	24.0	17.1	5.4	18.5	7.4
T_5 (treated with HA+ planted by Faba bean)	Dissipation amount	1045.0	400.0	450.0	132.5	267.5	195.0
	Dissipation ratio (%)	39.8	15.2	17.1	5.0	10.1	7.4
****		PAHs (mg.kg^{-1}) after 90 days					
		D_p	D_{en}	D_m	D_{pa}	D_{pp}	D_{HA}
T_4 (treated with HA+ planted by Alfalfa)	Dissipation amount	1695.0	610.0	710.0	162.5	447.5	375.0
	Dissipation ratio (%)	64.6	23.2	27.0	6.2	17.0	14.2
T_5 (treated with HA+ planted by Faba bean)	Dissipation amount	1175.0	90.0	710.0	0.0	90.0	375.0
	Dissipation ratio (%)	44.8	3.4	27.0	0.0	3.4	14.2

- *D_p: dissipation amount of PAHs (mg.kg^{-1})in planted soil;*
- *D_{en} : dissipation amount enhancement of PAHs (mg.kg^{-1})in planted versus unplanted soil.*
- *D_m : dissipation amount of PAHs (mg.kg^{-1}) by initial microorganisms*
- *D_{Pa}:(plant uptake) accumulation amount of PAHs (mg.kg^{-1}) or removal by plant.*
- *D_{pp}: the loss of PAHs (mg.kg^{-1}) in planted soil by plant promoted biodegradation.*
- *D_{HA}: dissipation amount of PAHs (mg.kg^{-1}) by HA addition to soil contaminated.*

Conclusion

Addition of HA as a bio stimulation to Phytoremediation process in planted contaminated soil, which enhanced the plant growth and microorganisms activity in rhizospheric soil. Thus the dissipation amount and dissipation ration of PAHs pollutants in planted soil were affected by three principal factors and its interaction through Phytoremediation process, they are plant cultivated, microorganisms in rhizosphere and HA addition. Pot experiments present an approach model to quantify the role of each factor alone in bioremediation process or phytoremediation technique to degrade total PAHs in contaminated planted soil. Results indicate that Alfalfa plant was the preferable plants used to clean up the soil contaminated by crude oil through phytoremediation technique which gave the maximum dissipation ration of PAHs (64.6%) at 90 days after sowing.

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