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BIOASSAY OF CRUDE OIL TOXICITY IN SOIL AND *VECIA FABA* L. PLANT

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Abstract

Effects of crude oil contamination on properties of soil and some growth characteristics of broad bean *Vicia faba* L. plant were carried out. The seeds of broad bean were planted in plastic pots under different levels of crude oil (0, 10, 50, 75 ml/kg soil) for 60 days. The results were showed significantly decreased in the water content of soil after five days of irrigation at 75 ml/kg from 0.15 to 0.03 g/g of soil. Electrical conductivity increased from 1100 to 1260 µcm / cm while pH values were changed from alkalinity (8.3) in the control group to acidity (6.6) under high treatment with increasing of organic matter from (0.72-2.6%) at low and high treatment. Microbial biomass and urease activity of soil were significantly reduced after 60 days under high treatment of crude oil about (11.2 µg C-CO_2/g soil) and (10.6 µgN-NH_4) respectively in the experiment end. However, it was recorded (26.6 µg C-CO_2/g soil(and (16.2 µgN-NH_4. g soil.2h1) in control group also respectively after same period because of the toxic effect of crude oil which was also detected in some parameter in broad bean plant included germination rate, length, dimension of stem, dry and fresh weight of root and shoot. Statistical analysis indicated that significant differences between the treated plants. However, all parameters were not significantly different in 10 ml treatment because of stimulation and encourage effect of soil degraded biota under this concentration in breakdown of oil component and low toxicity of hydrocarbons.

Key words : *Vicia faba*, crude oil, microbial activity, urease.

Introduction

The problem of the organic pollution is a one problems affecting in the vitality of the agricultural land and their productivity, particularly persistent organic pollutants (POP), which have long-term toxicity in soil such as crude oil hydrocarbons (Kayode and Oyedeji, 2012; Pozo *et al*., 2012). The oil industry is spread over large areas of the Iraq south region causing expansion of pollution with the increasing number of oil wells and expand tanker piping in addition to the destruction and burning of oil wells and refinery plants during repeated wars and terrorist operations. Only during 2006 the amount of oil leakage (565149) M^3 and dry & liquid gas leaked (3560) tons by carrier pipe bombing 113 in seven provinces had more accidents in the provinces of Baghdad, Basra, Kirkuk and Salahuddin (The Iraqi Ministry of the Environment, 2006). There are different routes of oil and derivatives it to reach the soil as a direct oil spill, the oil industry and their products as well as rain and accident also wind can transfer the soil and irrigation polluted water.[1] The petroleum hydrocarbon as critical components of crude as a primary source of energy as well as asphalt and non-hydrocarbon compounds (Shaopeng *et al*., 2013).

When hydrocarbons reach to soil surface tend to accumulate between soil particles causing reduction of permeability of gasses and water then the viscosity of the mixture of hydrocarbons may cover the surface of soil particles and changing the water holding capacity and binding of clay minerals (Morgan and Atlas, 1989) significantly. The soil has the capacity adsorption agent to hydrocarbons suggested that increasing availability and duration of their degraded by evaporation, photooxidation, and biodegradation. Where horizontal distribution extent the polluted region and facilitated losing of low molecular weight hydrocarbons by evaporation and photooxidation (Zhang *et al*., 1995; Alber, 1995).

The studies indicated that petroleum hydrocarbons, such as benzene, phenol and aromatic hydrocarbons having multiple damages not only for plant growth and development but for the whole of the characteristic and biological activities of soil (Peng *et al*., 2009) such as pH, soil oxygen, nutrients availability, water holding capacity and gas exchange because of these compounds fill soil particles space which is negatively reflected in the density and species of the soil microbes (Wyszkowska, 2000; Gagoi *et al*., 2003; Maliszewska-Kordybach *et al*., 2007; Singh *et al*., 2005). Moreover, petroleum hydrocarbons in soil has negatively affected ammonification and nitrification reactions and minerals absorption especially nitrogen where organic carbon to nitrogen ratio will be change (Amadi *et al*., 1996; Rimowsky *et al*., 1998). Therefore, a high concentration of petroleum hydrocarbons in the soil can be toxic to photosynthesis, nutrient assimilation, and other plant processes then vegetative biomass reduction (Peng *et al*., 2009; Nie *et al*., 2011; Dowty *et al*., 2001).

Labud *et al*. (2007) found that crude oil and its derivatives in soil have impact of microbial activity and nutrient cycle especially in sandy soil as well as its impact on different soil properties including microbial community composition, soil resources consumption and soil enzymatic activities such as Dehydrogenase, Catalase, urease, and invertase so, these enzymes used as bioindicators in soil degradation because of their sensitivity to pollutants (Stanislaw *et al*., 2004; Achuba and Peretiemo-Clarke, 2008).

Visser and Parkinson (1992) and Dick and Tabatabai (1992) indicated that measurement of the changing in soil enzymes effectiveness are important indexes about the alteration of biological activity of the soil due to easily measure of enzymatic activity and sensitive enzymes to soil properties which associated with increased of added organic carbon to the soil is a source of energy and nutrition for producing soil microbes.so organic matter work as a barrier to protease enzymes in degradation of an enzymatic protein of other enzymes (Gianfreda *et al*., 2005; Bello *et al*., 2014; Burns *et al*., 2013).

Microbial biomass is total biomass of soil microbes which related all biological transformations then determined soil properties and nutrient content (Broos *et al*., 2007). Thus, it is a measure of the vitality of the soil so that the biomass measurement gives essential indicators about the species and activity of soil microbes especially with specific organic materials such as petroleum hydrocarbons where was considered as early evidence of environmental stress of the soil by diagnostic and determination of their enzymatic activity (Dick and Tabatabai, 1992; Eibes *et al*., 2006; Brohon *et al*., 2001).

In plants, hydrocarbons can be covering the roots then prevent or reduce gases exchange and water uptake thus reducing the absorption of nutrients and decrease of the root efficiency (Labud *et al*., 2007; Adam and Duncan, 2002; Serrano *et al*., 2009). On the other hand, it can obstruct some metabolic activities of embryos where permeate into the seeds through their impact on cellular membrane permeability, metabolites, and reduced respiratory rate for example in broad bean, oil products caused chromosomal aberration by damaging of nuclear material (Song *et al*., 2006; Liu *et al*., 2016; Iqbal, 2016).

In the current study, the primary goals are demonstrated of the toxic effect of different levels from crude oil in some physical and chemical soil properties and changing in microbial biomass, and enzymatic activity of urease then investigate the physiological and phenotypic traits of plants was affected as reduction of photosynthesis pigments, dry and fresh weight.

Materials and Methods

Preparation of polluted soil and cultivation

An experiment carried out by filling pots with sandy loam soil (Labud *et al*., 2007) then treated by three levels of crude oil that was brought from the general company refinery (Shanafiyah refinery branch) in Al-Qadisiya governorate [0, 10, 20 and 75 ml/kg soil] Quantities mixed well before packing in pots except control group was left without any addition . Five seed was planted per pot and watering equally and periodically to field capacity level then pots was lifted under shedding conditions. For recording data, such percentage of germination and chlorophyll pigments and other traits was sampling periodically.

The changing of soil properties

The soil sample was taken from pots treated for calculating values of water content, electrical conductivity and pH after different periods of soil saturated extract using a pH meter and EC-meter according to method of Black (1965b). Organic matter was estimated by wet oxidation methods by potassium dichromic solution (1.0 m) with sulfuric acid and titration with ferrous sulfate using Diphenyl Amin as reagent (Walkley and Black, 1934).

Fumigation-IncubationTechnique, as described in Schinner *et al*. (1996) was conducted to estimating microbial biomass by CO_2 as C extractable in fumigated soil extracts minus extractable C in non-fumigated soil extracts then vessels of NaOH 0.1 M titrated with 0.1 M HCl to determine all the CO_2 trapped.

Urease activity was estimated according to the described method in Tabatbai and Bremner (1972) by incubation of 5 g soil sample for 2h under 37^{0}C with the substrate (0.2 M urea solution 1 ml) and toluene 0.2 ml, and distillation and titration determined tris buffer pH=9. The ammonium N with diluted sulfuric acid. HCL and boric acid as reagent then the results were expressed as ìg NH_4-N g-1 $2h^{-1}$ of dry soil.

Plant characters studied were included percentage of germination, Diameter & Height (cm), Fresh and dry weights (g) of *Vicia faba* plants (root and shoot) under different levels of crude oil upon maturity. Pigment photosynthesis was extracted in 80 % acetone and calculated according to Lichtenthaler (1987). Electrolytes permeability percentage was estimated according to Dresler *et al*. (2014).

Statistical analysis

All results were analyzed by one-way ANOVA methods using SPSS statistical software. The least significant difference value (LSD) was used to identify any significant differences among the means of the treatments where (p-value $\leq$ 0.05) are considered significant.

Results and Discussion

The physical-chemical properties of contaminated soil by different levels of crude oil (0, 10, 50, 75) ml/kg soil were affected. The mean of soil moisture content for five days after watering to saturated level (table 1) reduced from (0.15-0.1g) in control group to (0.01-0.03 g) under treated it by 75 ml/kg soil after 60 days. Statistically there were significant difference recorded between treatments due to reduction in water holding capacity by creation of insulating layer of oil components of soil particles allow water to leak down rapidly maybe because of hydrophobicity of oil compounds, which formed membranous layers surrounded of soil particles as well as decrease of interfacial tension at the oil-water boundary, then solubility of hydrophilic soil particles which reduced and obstruct their absorption and bioavailability to soil biota and plant roots (Obasi *et al*., 2013).

pH values of contaminated planting soil by broad beans were also changed after 60 days of treatment to fell from 8.6 before treated to 6.4 under 75 ml/kg of soil, while it was slowly reduced in the control group (table 1) to 7.8 may be affected naturally by biological processes. However, a high concentration of organic oil compounds can be decomposed by a microorganism to produce organic acids which caused changing in pH values by consumption of hydrocarbon as an energy source to produced long-chain organic acids and unsaturated hydrocarbons that mean resulted in oil and water emulsions then reduction of pH level (Atlas, 1977). On the other hand, this may lead to negative effects of sensitive soil microbes, especially which have a sensitivity to specific toxic compounds concentration. Sexstone and Atlas (1977) found that fatty acids accumulation by biodegradation of hydrocarbons was toxic to microorganisms as well as pH value decreasing in the medium will be affected the acidity of outside and inside cells and enzymatic system.

Because of petroleum polluted of soil the concentration of ions was clearly changed through the increase in electrical conductivity values (EC) from 650 - 680 µs/cm in control while recorded 1100-1280 µs/cm at 75 ml/kg level respectively may be due to the various ions and heavy elements such as nickel, lead and vanadium in petroleum crude content or by soil and irrigation. However, the solubility of those ions in the soil solution decreased gradually when soil treated by 75 ml/kg according to the type of soil, pH, water content and microbial activity, while bioavailability of plant nutrients will be affected according to that. These results are consistent with Adam and Duncan (2002) that reported the availability of phosphate and nitrogen decreased dramatically in soils affected by oil pollution. Wherever, The decomposition of additive organic matter such petroleum will be change in chemical, physical or biological properties of soil through various oxidation processes unusually low molecular weight compounds which start by oxidation normal Alkanes and Alkenes to solid components (Essien and John (2010). In current study organic matter in soil decreased gradually after 8 weeks from 0.72-2.6% under 0 and 75 ml of oil treatments respectively, suggested that additive petroleum organic matter was gradually converted by different biochemical processes to soluble matter caused changing of pH, micronutrients, and organic matter than soil density, water holding capacity and nutrient bioavailability will be improved (Lichtenthaler, 1987). The remaining petroleum hydrocarbons are complex molecules requiring various operations will leave a long-term effect on agricultural soils. So, Marý´n (2004) found that organic matter in soils refineries reached 11% after 60 days, then the decreased to 4% by microbial consumption and degradation.

Microbial biomass alteration

The changes measurement of microbial biomass and evolution rate of co_2 and Urease activity used as an indicator of the degree of petroleum polluted soil (Eibes *et al*., 2006; Brohon *et al*., 2001). Thus, the current study demonstrated that the increase of microbial mass in soil

Table 1 : The range and Standard deviation of changing in some soil properties, microbial biomass (µgC-CO_2/g soil) and urease activity (µgN-NH_4.g soil.2h^{-1}), of soil contaminated with different levels of crude oil upon maturity of *Vicia fab*a.

Soil properties	Units	Treatments (ml)				LSD values
		0	10	50	75	
Water moisture	g/g soil	0.15-0.1±0.02	0.16-0.09±0.029	0.14-0.05±0.04	0.15-0.03±0.058	-
Electrical conductivity	µs/cm	650-680±60.2	930-1050±50.6	1050-1120±30.9	1100-1260±67.3	260
pH	-	8.3-7.6±0.41	7.9-7.6±0.12	7.7-7.2±0.26	7.2-6.6±0.34	1.2
Organic matter	%	0.72±0.18	0.66±1.3	0.93±1.7	2.6±1.85	1.4
Microbial biomass	µg C-CO_2/g soil	20.4-26.6±2.8	3.4-22.7±8.1	6.6-12.4±3.5	4.6-11.2±3.2	2.7
Urease activity	µgN-NH_4.g soil.2h^{-1}	14-16.2±0.8	11.4-20.8± 3.5	6.6-11.8±1.9	4.7-10.6±2.4	2.4

polluted 4.6-11.2 µg c-co_2/g soil according to CO_2 evolved when treated with 75 ml/kg soil while ranged 24.4-26.6 and 3.4-22.7 µg c- co_2/g in control and 10 ml treated group. The statistical analysis pointed to significant differences in 10 ml and a control treatment with others after 14 days of incubation may be to soil organic matter and stimulation of non-sensitive microorganisms which consume organic carbons as an energy source (Margesin *et al*., 2000). So the CO_2 evolution rising with increased microbial activity after the incubation period. This is consistent with Atlas (1977), Sexstone and Atlas (1977). Many studies indicated that microorganisms density that consumed hydrocarbons increased rapidly in small concentrations. However, petroleum added to soil caused the selectivity decline in the activity of soil microorganisms during the first hours with absent to sensitive microorganisms then this response will be caused variation in microbial diversity and biomass with depended to petroleum level and species of an aromatic hydrocarbon. Likewise, the biodegradation of saturated hydrocarbons will be increased under low concentrations of oil while high degree of pollution will increase breakdown of aromatic and asphalt (Dibble and Bartha, 1979), this variation linked to density and structure of microbial community which consumed hydrocarbons under pollution condition and their proportion for total microbial percent which may be reached to 100% in polluted ecosystems while reduced to 1% in non-polluted sites (Atlas, 1981).

The enzymatic efficacy of petroleum compounds showed declines after 60 days reached to 0.03 (µgN-NH_4.g soil.2h^{-1}) of high treatment, while it recorded (0.82µgN.g soil.2h^{-1}) in control which statistically different with other treatment at the experiment end may be due to decreased of urease producers biota under overdose of petroleum pollution then increase gradually with increasing of heterotrophic microbial community according to sensitive species of bacteria, toxicity of petroleum derivatives and their proportion of biodegradable compounds (Margesin *et al*. (2000). So the addition high dose of gas oil and diesel inhibited urease activity in ending of incubation period while increased gradually under small dose of petroleum hydrocarbons (Wyszkowski and Wyszkowska, 2005; Lipinska *et al*., 2013). The interaction between petroleum compound and enzymatic activity due to the occupation of enzyme active site on cell surface because of their hydrophobicity and lipophilicity compounds as membrane prevent organic and non-organic molecules as a substrate to attachment of enzyme active sites. However, biosurfactants have been made by some resistance microbes as amino acid, carbohydrate and lipid can be enhance some biotransformation of hydrocarbons to complete oxidation process it. So, the enzymatic activity of microbes will be increased significantly by growing of microbial community and when replacing the water surrounding molecules replaced by less polar solvents (Labud *et al*., 2007; Serrano *et al*., 2009).

The deterioration of plants

The physiological and morphometric traits included the percentage of germination, electrolytes permeability rates, the thickness of stems (cm) fresh and dry weights were recorded of Vicia faba plants which grown under different levels of crude oil even flowering stage (table 2). Germination rate decreased significantly to 30% under treatment of 75 ml/kg from crude oil while recorded 90% in control group with significant differences in statistical analysis (LSD=11.4) may be because high toxicity of petroleum hydrocarbons of broad bean seedling which affected cellular division in apical meristem of roots that caused chromosomal abbreviation in the resulting cell (Song *et al*., 2006). Thus, this interruption negatively reversed in the reduction of plants lengths and diameters rates to 7.6 and 1.2 cm at the high treatment while recorded 24.6 and 2.2 cm for the control group, respectively. Fresh and dry weight rates also decreased to 0.23 and 0.06 g respectively in the root While recorded 8.4 & 0.16 g for shoot under 75 ml/kg treatment. In contrast, they recorded (1.6 & 2.3) for root and 42.8 &

Table 2 : Percentage of germination, pigment photosynthesis, permeability rates higher plants and diameter & height (cm) fresh and dry weights (g) of *Vicia faba* plants soil treatment ruptures with different levels of crude oil upon maturity.

Crude oil amount	Germination (%)	Permeability (%)	Height (cm)	Diameter (cm)	Dry weight of root (g)	Fresh weight of root (g)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Photosynthesis pigments (mg/g)		
									Chl. a	Chl. b	Carotenoids
0	90%	35.77	24.6	2.2	1.6	2.3	42.8	2.3	1.4	0.3	0.24
10	55%	30.59	31.7	1.6	0.17	0.6	35.6	0.8	0.9	0.25	0.2
50	42.5%	59.21	8.8	1.8	0.2	0.5	20.8	0.4	0.65	0.22	0.16
75	30%	60.8	7.6	1.2	0.067	0.5	3.4	0.2	0.3	0.15	0.1
LSD	11.4	0.004	0.052	0.023	0.06	0.23	8.4	0.16	0.12	0.09	0.02

2.4 for a shoot in control group, respectively. Permeability of electrolytes also affected by 60% at high treatment after they were 35.77% in the control group may be due to interference of hydrophobic petroleum compounds with cellular membranes structure especially the low molecular weights of polycyclic aromatic hydrocarbons, which change their permeability and thus disrupt the ionic balance and barrier properties as well as inhibitory effect in many metabolic processes within plasma membrane and chloroplast such photosynthesis activity and free radical generation (Kreslavski *et al*., 2017; Alwan, 2015). Leaves content of photosynthesis pigments also reduced with increasing of petroleum polluted of soil to (0.3, 0.15 and 0.1) mg/g fresh weight of leaf under 75ml/kg soil for chlorophyll a, chlorophyll b and carotenoids (table 2) while recorded (1.4, 0.3 and 0.24 mg/g) in control group respectively. These results were agreement with those obtained by Meng and Chi (2015) that stated highest concentration of PAHs (more than 180 mg/ kg^{-1} dry sediment) was toxic of *Potamogeton crispus* in the reduction of chlorophyll content and root morphology and biomass. Jajoo *et al*. (2014) reported that *Arabidopsis thaliana* plants grown for six weeks under PAH-treated soil was undergone a reduction in content and composition of photosynthetic pigments, these effects may be caused changes in protein composition and photosynthetic performance. Moreover, cytotoxicity of petroleum hydrocarbons on root tip cells of *Vicia faba* caused micronuclei formation and chromosomal aberration such chromosome loss, chromosome break and others (Iqbal, 2016; Tabatabai and Bremner, 1972).

Conclusion and Recommendations

Petroleum pollution of soil and combustion products of oil and derivatives have considerable interest from many researchers due to their toxicity, teratogenicity and carcinogenicity of petroleum hydrocarbons to human, animal, and plant with the expansion of oil industries and use of fossil fuels. Thus, crude oil chemically have organic nature so adding it to soil caused a change in the physical and chemical properties of soil and biotic nature, however, the critical aspect is toxic effects of petroleum hydrocarbons and its ability to penetrate and accumulate into the cells and tissues of biota can interact with crucial metabolism pathway caused many changes in microbial structure and biodiversity of soil biota.

Enzymatic activity considered as early evidence of environmental stress of soil (Dick and Tabatabai, 1992; Eibes *et al*., 2006; Brohon *et al*., 2001). Especially alkane oxygenase, integral membrane alkane hydroxylases and another enzyme that related with biosurfactants production such rhamnolipid, lipopeptides, glycolipids, and others also as bioindicators to microbial degradation of petroleum compounds as well as the study of microbial community structure after complete degradation processes and determined of resistant species and strain with a different period. So arguably, it can minimize the toxicity of petroleum hydrocarbons by encouraging the growth of microbes to bioremediation for these compounds especially in surrounding land of all petroleum activates by an agricultural application such, plenty irrigation, tillage, organic fertilizer and physical methods like solar heating to land reclamation.

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