Assessment the Impact of Oil Refinery Residues on Soil Enzymatic Activity

Dalshad.A. Darwesh
Dept. of Environmental Sciences
University of Salahaddin-Erbil
Erbil,Iraq
dalshas.darwesh@su.edu.krd

Amel. F. Ibrahim
Dept. of Environmental Sciences
University of Salahaddin-Erbil
Erbil,Iraq

Abstract

A laboratory experiment was conducted to assessment the effect of oil refinery residues on enzymatic activity in two textured soils. The both soil samples sandy loam and clay treated with crude oil residues levels (0, 5, 10, 15, 20, 25, 30 ml / 250g soil) and water was added to bring the soil moisture to 60% of the field capacity and the influence of the crude oil residues on the activities of soil enzymes catalase, dehydrogenase and urease were determined. The results show that the addition of oil residues significantly stimulated the activity of urease and catalase enzymes but inhibited the activity of dehydrogenase. The highest values 160.767 µg $N.g^{-1}$ soil and 5.648 µg H_2O_2 $.g^{-1}$ soil for the activity of urease and catalase respectively were recorded in 15ml oil residues, while the lowest activity of dehydrogenase 27.218 µgTPF$.g^{-1}$ soil was found in sample receive (30ml oil residues) . However the results show that the higher values of dehydrogenase and urease activity were recorded in clay soil while higher catalase activity was recorded in sandy loam soil which attained 5.848 µg H_2O_2 $.g^{-1}$ soil.

Keywords: catalase, dehydrogenase, urease, soil texture, crude oil residue

Introduction

Crude oil is one of the most important natural resources in the world; it is used as raw material in many industries, including the refinery-petrochemical industry [1]. All soils contain a group of enzymes that determine soil metabolic processes which, in turn depend on its physical chemical, microbiological, and biochemical properties [2]. A variety of methods were developed to measure soil biological activity. All these methods are not suitable to provide an accepted result, but they give relative information about the ecological status of the soil. Some enzyme activities serve as a measure of the microbial intracellular activity (e.g. dehydrogenase, catalase), which typically reflect general

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microbial activity in soil, While others reflect extracellular activity (e.g. phosphatase, urease) in the soil and therefore can be used as toxicity tests [3]. Crude oil pollution adversely affects the soil ecosystem through adsorption to soil particles, provision of an excess carbon that might be unavailable for microbial use and an induction of a limitation in soil nitrogen and phosphorus. These processes which affect drastically soil enzymatic activities [4]. Several studies were carried out around the world on the effect of crude oil residues on (dehydrogenase and urease), (catalase and dehydrogenase), (dehydrogenase) and (urease and catalase) enzyme activity by [5 and 6], [7 and 8], [9] and [10] respectively. In Kurdistan region with rapid increasing of economic and industrial development, the number of the oil refinery and other related industrial activities ever-increasing has been resulted in severe environmental pollution. In 10 recent years commercial oil production widespread in or near agricultural areas, Therefore large amount of industrial pollutants that are released in to the environment, which decrease the physical, chemical and biological properties of soil, This can reduce the soil fertility, influences and modifies soil microbial community composition, biogeochemical cycles, and decline the soil capacity to support plant growth. In addition very few and scattered study is available to investigate the status of the environment around the refinery locations of Kurdistan region, thus the main objective of the present study is to determine the effect of adding crude oil residues in deferent levels on enzymatic activities in deferent textured soil.

Materials and equipment

A laboratory experiment was conducted to assessment the effect of oil refinery residues on enzymatic activity in two textured soils. The both soil samples sandy loam and clay were chosen based on the courses and finesses, then both soils treated with crude oil residues levels (0, 5, 10, 15, 20, 25, 30 ml / 250g soil) and sterilized distilled water was added to bring the soil moisture to 60% of the field capacity and the influence of the crude oil residues on the activities of soil enzymes (catalase, dehydrogenase and urease) were determined.

Methods

A. Soil analysis

The soil samples were collected from Iski-kalak and Grazaban areas in Erbil governorate of Iraq Kurdistan region. The samples were taken from surface layer 0 – 30 cm and sieved by 2mm screen, then analysis to determination some physical, chemical, and biological properties according to methods described by [11, 12, 13, 14, 15, and 16].

B. Soil enzymatic activity

Urease activity. Urease activity was assayed by method as described in [17] 0.25 ml toluene, 0.75 ml citrate buffer (pH, 6.7) and 1 ml of 10% urea substrate solution were added to (1g) soil and the soils incubated for 3 hrs at37˚C. The formation ammonium during decomposition of urea was found out spectrophotometricaly at 578 nm and the results were expressed in μg N g^{-1} dry soil.

Dehydrogenase activity. Dehydrogenase was determined following method as mentioned in [18] after soil incubation with 2, 3, 5 triphenyltetrazolium chloride (TTC) and the dehydrogenase activity was estimated by measurement of triphenylformazan (TPF) absorbance at 485 nm. The values of dehydrogenase activity are expressed as μg TPF g-1 dry weight soil

Catalase activity. Catalase activity was determined by the method as described in [7]. One hundred ml of phosphate buffer, pH 7.4, was added to 10 g of soil and stirred vigorously. The soil suspension was filtered using cheesecloth. The filtrate was centrifuged for 10 min to obtain the supernatant. The soil extract (0.5 ml) was added to the reaction mixture containing 1 ml of 0.05 M phosphate buffer (pH 7.5), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for 3 minutes. The reaction was terminated after 3 minutes by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). All tubes heated for 10minutes in boiling water and the absorbance was read at 610 nm. Catalase activity was expressed as μg $H_2O_2.g^{-1}$ soil.

C. Statistical analysis

Data was statistically analyzed using SPSS software program. All data results are expressed as mean value. The difference among the means of soil texture and oil residue levels was compared by applying Duncan multiple comparison and least significant difference test (L.S.D) at (5%) level of significant [19].

Results and Discussion

Soil enzyme is one of the principal soil components, in spite of the minimum quantity, their functions are very important [3]. Petroleum-derived products disturb the soil structure and modify its physicochemical properties. They also affect the biological properties of the soil by altering the populations of particular microorganisms. This, in turn, influences the soil enzymatic activity [20]. Data present in table (2) indicated that soil textures were significantly at probability ($p \leq 0.05$) affected catalase and dehydrogenase activities and non-significantly affected the activity of urease enzyme. The highest values (135.943 μg N.g-1 soil, 52.065 μgTPF.g-1 soil) and (5.848 μg H2O2 .g-1) for (urease, dehydrogenase) and (catalase) were recorded from clay soil (S2) and sandy loam soil (S1) respectively. While the lowest values (121.612 μg N.g-1, 35.245

µgTPF.g-1) and (3.784 µg H2O2 .g-1) for above mentioned enzymes were recorded in the (S1) and (S2) respectively. These results indicated that the soil textures influence the enzymatic activity, and it is greater in clay soil than sandy loam soil and could be interpreted on the basis that the clay has large specific surface area, high number of available active sites.

However, clay soil contained more organic matter, and higher pH and EC than the sandy loam soil. The organic matter and soil pH had a direct positive effect on urease and dehydrogenase activity; while catalase activity was closely related to soil EC than the other physical-chemical properties. However, the clay soil has small pores for instance hold water well and conduct heat that affects the activity of soil microorganisms. This result and interpreting in accord with those reported by [21] showed that the variation of soil enzyme activity was significantly attributable to differences in soil texture, carbon, nitrogen and phosphorus content, bulk density, water holding capacity, moisture content and soil pH. The findings demonstrated that soil organic carbon, total nitrogen, available phosphorus and clay content are the important determinants for dehydrogenase activity and moderate correlation with soil pH, moisture content and water holding capacity.

Table 2. Effect of soil textures on enzymatic activity

Soils	Enzymatic activity		
	Dehydrogenase µgTPF.g-1 soil	Urease µgN.g-1 soil	Catalase µg H2O2 .g-1 soil
Sandy loam (S1)	35.245	121.612	5.848
Clay (S2)	52.065	135.943	3.784
LSD 5%	13.891*	29.504	0.925*

The data present in table (3) indicated that oil residue levels were significantly at ($p \leq 0.05$) affected enzyme activity in studied soils. The highest values (160.767 µg $N.g^{-1}$ soil, 5.648 µg H_2O_2 $.g^{-1}$ soil) and (70.825 µgTPF.g^{-1} soil) for (urease, catalase) and (dehydrogenase) were recorded in (15ml. 250g-1soil) and (control) treatment respectively. While the lowest values (86.992 µg $N.g^{-1}$ soil, 4.124 µg H_2O_2 $.g^{-1}$ soil and 27.218 µgTPF.g-1soil) for above mentioned enzymes were recorded in the (25 ml. 250g^{-1}soil, control, and 30 ml. 250g^{-1}soil) treatment respectively. These results indicate that the effect of oil residues levels on the enzymatic activity of soil depended largely on the degree of contamination. Oil residues irrespective of the soil texture, significantly stimulated the activity of urease and catalase enzymes but inhibited the

activity of dehydrogenase. The obatin result in this study indicated that highest values of urease and catalase activity were observed in 15ml of oil residues application. This result may be explained on the basis that oil residues are mixture of several different chemicals containing low and high molecular weight and decomposition products and these compounds may be used by soil organism as source of carbon and energy, thus the enzymatic activity increase with increase the concentration of such compound, another cause of the increased soil enzyme activity might be due to biodegradation of organic compound that carried out by microorganisms.

Table 3. Effect of oil residues levels on enzymatic activity

Oil levels	Enzymatic activity		
	Dehydrogenase gTPF.g^{-1} soilμ	Urease gN.g^{-1} soilμ	Catalase μg H_2O_2 .g^{-1} soil
L0=0ml	70.825^{a}	126.858^{c}	4.124^{a}
L1= 5ml	41.106^{abc}	141.483^{b}	4.924^{a}
L2= 10ml	66.441^{ab}	143.000^{b}	4.703^{a}
L3 =15ml	33.610^{bc}	160.767^{a}	5.648^{a}
L4 =20ml	32.807^{bc}	142.567^{b}	4.796^{a}
L5=25ml	33.576^{bc}	86.992^{d}	4.872^{a}
L6 =30ml	27.218^{c}	99.775^{d}	4.646^{a}

The same letter mean no difference, while different letter mean significant difference at $p \leq 0.05$.

The stimulation effect reduced when residues of oil levels increased to 25ml and 30 ml in the soil. These results and explanation accord with those recorded by [22]. They indicated that addition of oil up 5.0 g and 10 g to soil was usually not toxic to the microbial activity since hydrocarbons present in oil could be serving as an additional carbon source for microbial growth. The results of studies carried out by [23] showed that the soil contaminated with 9 g diesel oil kg^{-1} soil, the activity of urease was stimulated, while when the soil polluted with 24 g of diesel oil kg^{-1}, the enzyme activity was inhibited. This result and explanation supported by those found in figures (1 and 2) which revealed that there is significant positive correlation between oil residues levels and urease, catalase activity (r=0.834 and r=0.760) respectively. The negative and significant correlation (r=- 0.801) of dehydrogenase activity with oil residues levels is presented in figure (3). However, dehydrogenase activity in both soils treated by oil residues decreased by (61.570 %) over non-treated soils. The application of oil residues addition seemed to be the most toxic for the dehydrogenase activity; thus the addition of petroleum residues led to a decrease in dehydrogenase in comparison with the control. The relationship between the activity of dehydrogenases and levels of oil residue was reported by [24], they indicated that the oil products negatively affected the activity of dehydrogenase and the application of the highest dose of lead-free and lead petrol 6 cm3

kg^{-1} of soil resulted in reduction of dehydrogenase activity by 6.5 and 5.3 times respectively over control.

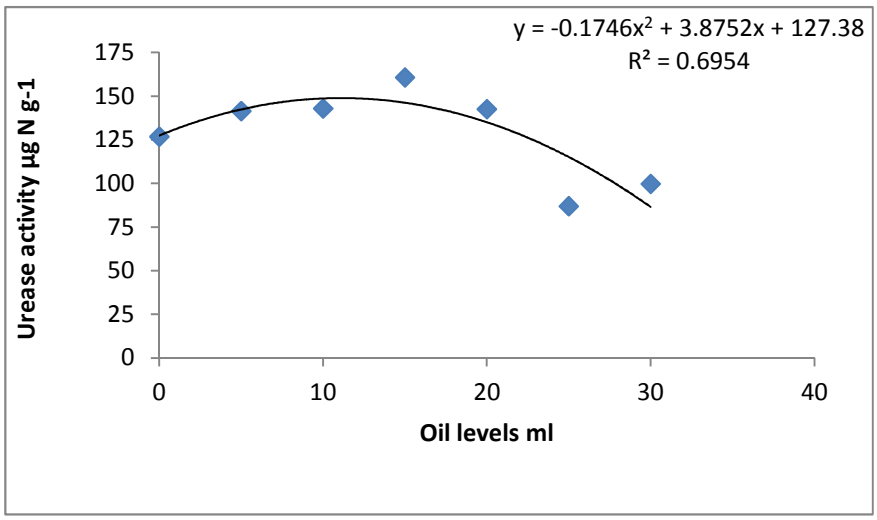

Figure 1. Relationship between urease activity and oil residues levels.

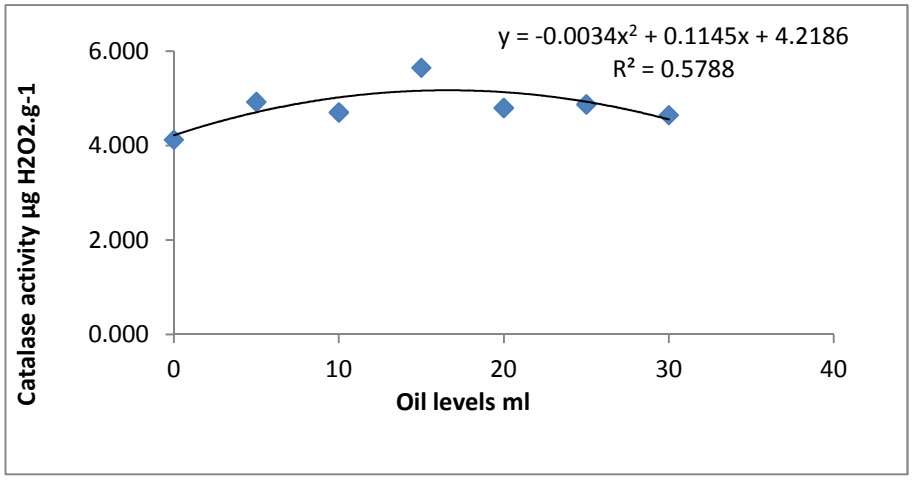

Figure 2. Relationship between catalase activity and oil residues levels.

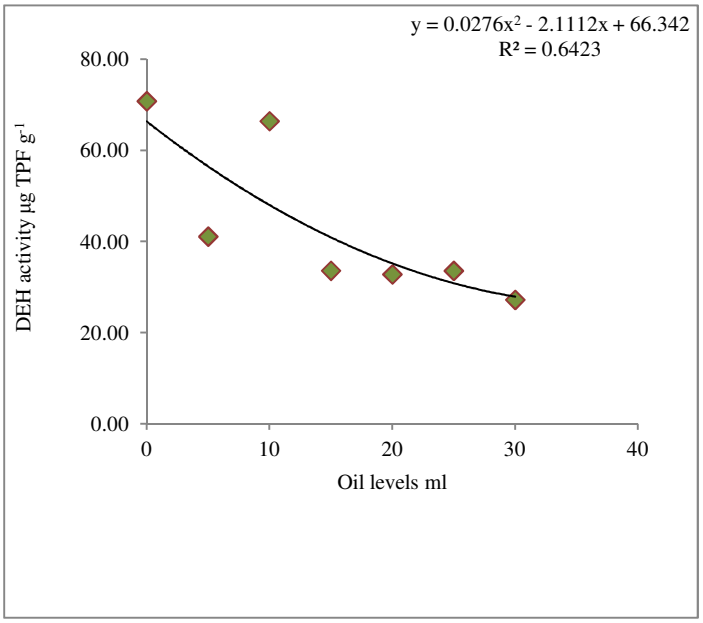

Figure 3. Relationship between dehydrogenase activity and oil residues levels.

Products negatively affected the activity of dehydrogenase and the application of the highest dose of lead-free and lead petrol (6 cm3. kg^{-1} of soil) resulted in reduction of dehydrogenase activity by 6.5 and 5.3 times respectively over control. The result in figure (4) show variation in urease activity under different levels of oil residues, the maximum increase of urease activity over control was (26.729%) produces by treatment that receive 15ml oil residues, beyond that the urease activity decrease the maximum decrease was (-31.426%) recorded from the application of 25ml oil residues over control. However the data analysis in figure (5) revealed that the catalase activity increase over control at all oil residues levels, the maximum increase was recorded at 15ml oil residues which attained 36.946% over control, whereas the dehydrogenase activity decrease with increase the oil residues levels, the maximum decrease was 61.57% produced by 30ml of oil residues over control figure (6).

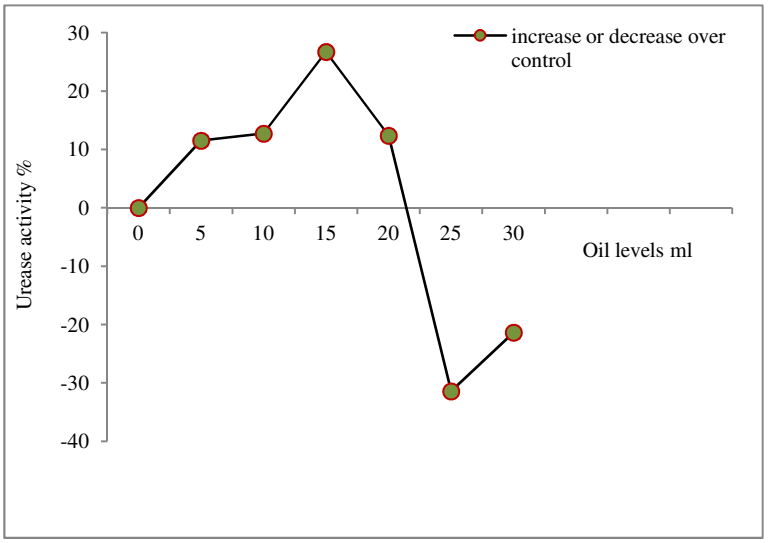

Figure 4. Variation in urease activity under different oil residues levels.

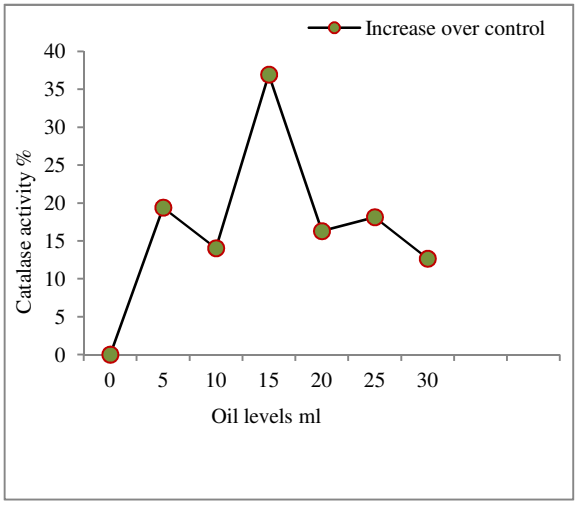

Figure 5. Variation in catalase activity under different oil residues levels**.**

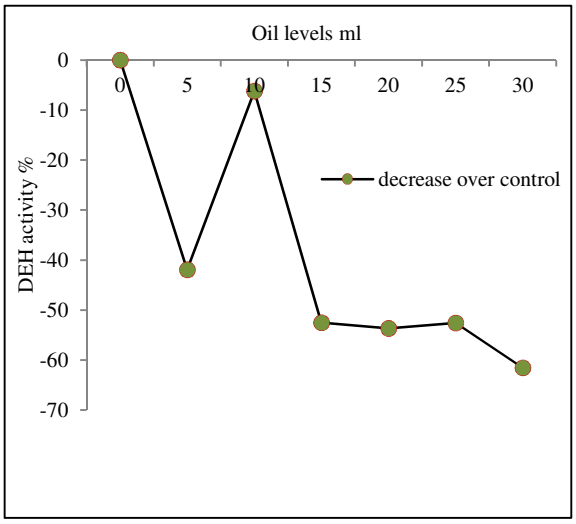

Figure 6. Variation in dehydrogenase activity under different oil residues levels.

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