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Effect of pollution with crude oil and used motor oils on the concentration of protein and carbohydrates in safflower and flax plants

Mostafa Thaier Ismail
Department of Biology, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq.
Email: mostafa.20esp38@student.uomosul.edu.iq

Hussein Saber Mohammed Ali Al-Rashedy
Department of Biology, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq.
Email: dr.husseinbio76@uomosul.edu.iq

***Abstract*---**This study was conducted to clarify the effect of soil pollution with crude oil, car, and generator used oil on the concentration of protein and carbohydrates in *Carthamus tinctorius* and *Linum usitatissimum* plants as the soil was treated with crude oil and used oils for car engines and generators at three concentration for each , which are(1, 2, 3)%. The results showed that the treatment of safflower and flax plants with used motor oil and crude oil at concentrations (3)%, respectively, led to a significant decrease in protein concentration in the shoots of the two plants, reaching (0.503, 0.175) mg/g dry weight, respectively. Then, treating the two plants with crude oil at the concentration (3)% led to a significant decrease in the carbohydrate concentration in the shoots of the two plants, reaching (1.110) and (1.100) mg/g dry weight, respectively, compared to the comparison treatment, which amounted to (3.805, 3.860) mg/g. Dry weight, while we note that crude oil was significantly more effective, as it led to a decrease in the protein concentration in the two plants, reaching (0.565) mg/g dry weight, and no significant differences were observed between the effect of oil and oils for engines of generators and cars. And used in the concentration of carbohydrates as the effect of the type of oil and the effect of the plant type, but the safflower plant significantly exceeded the protein concentration of the flax plant and reached (0.629) mg/g dry weight as the effect of the plant type.

***Keywords*---**crude oil, engine oil, flax, safflower, proteins, carbohydrates.

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Introduction

Soil pollution causes many environmental problems, and crude oil and its derivatives are among the most important of these pollutants, as they work to change the chemical, physical and biological properties of the soil . Oil pollution may have occurred from old and worn-out oil tanks and oil transmission pipelines , and natural leaks, as well as fires in oil wells, and the amount of pollution varies according to the properties of the soil and the chemical properties of the pollutants (Zahermand *et al.*, 2020). Oil pollutants lead to the deterioration of the soil's chemical, physical and biological properties and consequently restrict plant growth. It is productivity (Hung et al., 2020), and the reduced growth could be due to the lack of soil aeration due to the presence of pollutants, which reduces the concentration of nutrients, stops growth, and causes wilting and stunting. It has been proven Petroleum hydrocarbons stimulate oxidative stress, reduce growth, and distorted leaves, leading to necrosis of plant tissues and cells (Mukome *et al.*, 2020), in addition to the fact that the hydrophobic properties of hydrocarbons with low molecular weight hinder the effectiveness of the plant to absorb water and nutrients and thus negatively affect plant growth (Baoune *et al.*, 2018).

Proteins are biological molecules consisting of a series of amino acids linked together by peptide bonds. Although different proteins have similar amino acids, they have multiple and different functions. They are involved in almost all biological processes due to the wide variety of three-dimensional structures that make them up. They are included in the structural components of cells . Proteins are responsible for transporting materials to and from the cell across the cell membrane. Proteins catalyze the reaction (enzymes), and they are important in energy transfer, respiration, photosynthesis, cellular communication, molecular recognition, and gene expression (Kessel and Ben, 2018); it is known that the protein content is an important indicator of the physiological status of plants (Ma *et al.*, 2018), and many studies showed that the protein concentration decreased significantly with the increase in the oil concentration, including the decrease in the protein concentration in the *Mirabilis Jalapa* plant. With an increase in the concentration of crude oil in the soil (Ma et al., 2018), the study showed by Alzurfi *et al.* (2019) a significant decrease in the protein values of *Hydrilla verticillata* , plant when exposed to different concentrations of crude oil (0.2, 0.1, 0.05, 0.01)% for a period of 24 days.

Carbohydrates are a main source of energy, consisting of carbon, hydrogen, and oxygen, which plants manufacture and store as an energy source through the process of photosynthesis, from which the plant manufactures proteins and fats (Monalisa and Jain, 2015). Carbohydrates constitute about (60 to 80)% of the dry mass of the plant (Ghildiyal, 2021). One of the symptoms observed in plants growing in soil contaminated with crude oil and its derivatives , which causes a decrease in the carbohydrate content (Achuba and Ja-anni, 2018), oil and its derivatives have negative effects on the carbohydrate content in plants due to its effect on the plant pigments responsible for photosynthesis (Njoku *et al.*, 2018), which leads to a decrease in plant production ,and therefore a decrease in carbohydrate content, in addition to the effects Toxicity of heavy metals present in crude oil and its derivatives, which destroy the electron transport chain in the photosynthesis process, produce free roots , and affect carbohydrate metabolism by inactivating the enzymes responsible for carbohydrate synthesis (Khan *et al.*, 2015).

The study aims to:
Study of the effect of crude oil and used motor oils on protein and carbohydrate concentration in the shoot systems of flax and safflower plants.

Materials and Methods

Soil preparation

The soil of farms in the Zayouna district in Nineveh governorate was selected for experiments, where the surface soil was taken, dried with air, and then passed through a sieve with holes diameters of 2 mm. Cultivation was done in the Plastic House, and the tests were conducted in the scientific laboratories of the College of Education of pure Science Department of biology/University of Al Mosul.

Soil Analysis

The degree of electrical conductivity (E.C.) and soil organic matter were measured according to (Richard, 1954) and soil texture according to (Black and Hartge, 1986) method. The concentration of sodium and potassium was estimated using a Flame photometer device and exponent hydrogen P.H. meter, and the concentration of calcium, chloride, and Magnesium according to (Black, 1965) method. As in Table (1), in addition to estimating the field capacity of the soil.

Table (1) Physical and chemical properties of the soil before cultivation

The Estimation	**trait**
The texture	loam
Organic matter (%)	3.488
electrical conductivity (EC)	1899
pH	7.680
Estimation of some mineral elements (mg\kg)	
Na^{+}	72
K^{+}	120
Mg^{+2}	142
Ca^{+2}	257
Cl^{-}	83

Plastic House Experiments

Plastic house experiments were conducted to study the effect of pollution with crude oil and its derivatives on the concentration of protein and carbohydrates in the shoot parts of Flax (*Linum usitatissimum* L.) and Safflower (*Carthamus tinctorius* L.), and with three replications for each treatment in addition to the control treatment, as three treatments were prepared, represented by car engine oil and used generator engine oil in addition to crude oil. Each treatment had three concentrations of 1, 2, and 3%, and the experiment was implemented using plastic pots with diameters of 23 cm. Its height was 20 cm, and the capacity of each pot was 5 kg of soil.

Agriculture and Irrigation

The seeds were sown on 13/10/2021 by placing 20 seeds for each pot, then reduced after ten days of planting to only six plants in each pot; Pots were randomly placed in the plastic house's conditions and irrigated with water at the correct capacity (75)%. The amount of water added daily was controlled by using the scale, then the plants were harvested after 54 days.

Carbohydrate Estimation

The number of carbohydrates in the shoot was estimated according to the method (Herbert *et al.*, 1971), where the dry samples were crushed in a casserole with (10) ml of distilled water; after that, they were separated. The filter was removed from the sediment by using centrifugation, and the carbohydrates were estimated using the (phenol-sulfuric acid) method, then measured the visible density at wavelength (488) nm using a spectrophotometer

Protein estimation

The protein was estimated according to(Schacterale and Pollak, 1973) and modified by (Lowry *et al.*, 1951) by measuring the visible density at wavelength (640) nm using a spectrophotometer

Statistical analysis

The experiments were designed and statistically analyzed using the Completely Randomized Design (C.R.D) in factorial experiments (Anter, 2010). The significant differences in treatment rates were compared using (Duncan's New Multiple Range Test).

Results and Discussion

Estimation of protein conten
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Table (2) shows that the treatment of safflower and flax plants with motor oil and crude oil at a concentration of (3)% led to a significant decrease in the protein content of the two plants which mentioned above, amounting to (0.503, 0.175) mg/g of dry weight, compared to the comparison treatment, which amounted to (0.686, 0.643) mg/g of dry weight, respectively. This decrease may be because oil pollutants affect the availability of elements in the soil, including nitrogen .(Odiyi *et al.*, 2020) and thus affect the process of protein production; in addition to that, severe changes in the environment resulting from pollutants may lead to the modification of gene expression, which affects the process of protein production in plant cells (Haider *et al.*, 2021).

Table No. (2) Effect of treatment with crude oil and its derivatives on protein concentration (mg/g dry weight) for flax and safflower plants

treatments		control	Oil of generators			Oil of cars			Crude oil		
			%1	%2	%3	%1	%2	%3	%1	%2	%3
Plant type	safflower	0.686 a	0.642 ab	0.632 bc	0.557 de	0.630 bc	0.590 cd	0.503 fg	0.673 ab	0.637 abc	0.623 bc
	flax	0.643 ab	0.545 def	0.522 efg	0.491 g	0.555 de	0.545 def	0.483 g	0.587 cd	0.498 fg	0.175 h
Effect of oil type			0.590 a			0.579 ab			0.565 b		
Effect of oil concentration		0.665 a	0.605 b	0.571 c	0.472 d						
Effect of plant type	safflower		0.629 a								
	flax		0.528 b								

The rates with similar letters do not differ significantly at the (5%) probability level according to the test of Dunkin polynomial

It was noted from Table (2) that safflower was superior to flax plant in protein content and reached (0.629) mg/g of dry weight, and crude oil was more negatively affected than motor oils on protein content and reached (0.565) mg/g of dry weight. And that the concentration (3)% for all treatments had the most effect on the protein content of the two plants compared to the other concentrations, as it led to a significant decrease in the protein concentration and amounted to (0.472) mg/g of dry weight. The results are similar to what was found by (Achuba and Ja-anni 2018) that treatment of cowpea plant *Vigna unguiculata* with crude oil led to a decrease in the percentage of protein content in the plant, which amounted to (25.8)% of the comparison sample.

Carbohydrate content estimation

Table (3) shows that the treatment of safflower and flax plants with crude oil at a concentration of (3)% led to a significant decrease in the carbohydrate content of the two plants, which amounted to (1.110, 1.100) mg/g of dry weight, respectively, compared to the comparison sample, which amounted to (3.805, 3.860) mg/g of dry weight, respectively, and this decrease in the carbohydrate content may be attributed to the fact that oil pollutants with their heavy metal content negatively affect the photosynthesis of plants by reducing the content of chlorophyll and the absorption of the basic mineral elements of chlorophyll such as Magnesium, which affects the work of chloroplasts and the basic enzymatic processes in photosynthesis, thus reducing plant production and carbohydrate content (Alia *et al.*, 2015).

Table No. (3) The effect of treatment with crude oil and its derivatives on the concentration of carbohydrates (mg/g dry weight) for flax and safflower plants

treatments		control	Oil of generators			Oil of cars			Crude oil		
			%1	%2	%3	%1	%2	%3	%1	%2	%3
Plant type	safflower	3.805 a	3.543 a	2.425 ab	2.340 ab	2.870 ab	2.760 ab	2.375 ab	3.770 a	2.265 ab	1.110 b
	flax	3.860 a	2.977 ab	3.510 a	2.470 ab	2.943 ab	2.450 ab	1.110 b	2.990 ab	2.003 ab	1.100 b
Effect of oil type			3.116 a			2.772 a			2.613 a		
Effect of oil concentration		3.833 a	3.182 ab	2.569 b	1.751 c						
Effect of plant type	safflower	2.906 a									
	flax	2.761 a									

The rates with similar letters do not differ significantly at the (5%) probability level according to the test of Dunkin polynomial

In terms of the effect of the plant type and the effect of the three treatments (crude oil, generator engine oil, and automobile engine oil), no significant differences were observed between the two plants in the concentration of carbohydrate content in the vegetative tissues of the two plants. In terms of the effect of oil concentration, the results showed that treating the two plants with crude oil and motor oils at concentration (3)% was the most influential, negative, and significant on the concentration of carbohydrates contained in the two plants. It amounted to (1.751) mg/g of dry weight, and that this decrease may be because the growth of plants in soil contaminated with crude oil leads to a decrease in the area of leaves, which negatively affects the rate of photosynthesis and thus reduces the carbohydrate content in plants (Agbogidi *et al.*, 2007), as the treatment of *Parkinsonia aculeate* L. plant , with crude oil at concentrations (2, 4, 6)% led to a decrease in the carbohydrate content in the plant and reached (95.39, 89.39, 83.51) mg/g of dry weight, respectively, compared to the comparison treatment, which amounted to (111.74) mg/g of dry weight (AL-Tameemi *et al.*, 2020).

Conclusions

1 Oil residues with different concentrations affect the proteins and carbohydrates content of plants.
2 Plants vary in their ability to withstand different concentrations of oil pollutants.

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