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STUDYING THE POLLUTION WITH HEAVY ELEMENTS RESULTING FROM FUEL COMBUSTION FROM OIL WELLS IN THE PIGMENT OF TOTAL CHLOROPHYLL AND SOME CHEMICAL TRAITS OF DATE PALM LEAVES (*PHOENIX DACTYLIFERA* L.) HILAWI CULTIVAR GROWING IN BASRA PROVINCE, IRAQ

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Abstract

This study was conducted in five private orchards located in several areas of Basra provincel during the 2018 growing season. The study aims to know the effect of pollution with heavy elements resulting from the combustion of fuel from oil wells in Nahr Bin Omar region on some chemical traits of date palm leaves (Hilawi cultivar). The results of the study showed that the two locations (A and B) recorded a significant excelling in the total soluble carbohydrates in the leaf, the amount of total phenols in the leaf, and the amount of the amino acid (proline) in the leaf. The two locations (Control and D) also recorded a significant excelling in the concentration of total chlorophyll pigment in the leaves compared to the rest of the locations. Whereas, the Control location recorded the lowest value of the total soluble carbohydrates, total phenols, and the amino acid in the leaf, while the location (A) recorded the lowest concentration of total chlorophyll in the leaves.

Key words: date palm, total chlorophyll, proline, heavy metals, pollution, and oil wells.

Introduction

The date palm tree (*Phoenix dactylifera* L.) is considered a blessed fruit tree that originated in the Arabian Gulf and southern Iraq. Iraq represents the largest land cultivated with palm trees in the world. This tree has occupied great importance in terms of religious, economic, nutritional and environmental aspects of Arab people since the earliest times (Al-Bakr, 1972; Johnson, 2011), where the fruit of date palm contains a high percentage of sugars (44 - 88)%, fats (0.2 - 0.5)%, protein (2.3 - 5.6)% and 15 mineral elements such as iron, potassium, calcium, manganese, and others in addition to vitamins such as vitamin (C) and High percentage of fibers (6.4-11.5%) (Biglari, 2009). The fruits also contain plant dyes such as chlorophyll pigment, carotene, and anthocyanins, and they also contain a high percentage of important and necessary antioxidants for the body, such as phenolic substances such as cinnamic acid, ferulic and Fumaric, as well as flavonoids (Mansouri *et al.,* 2005). Heavy elements are considered dangerous pollutants, where their dangerous is being because they tend to accumulate in the soil and tissues of living organisms due to their non-degradation (Alloway, 1995; Dalman *et al.,* 2006). Despite the heavy elements that fall within the natural components of the earth's crust, humans have used them in many industries and agricultural applications, and their release led to environmental pollution (water, soil and air), thus their absorption by plant roots and subsequent accumulation for a long time in the food chain and its transfer from one organism to another. So it is a potential and influential threat to human and animal health, and this has caused great concern in the scientific and health community recently (Sprynskyy *et al.,* 2007). The results of the study conducted by (Zouari *et al.,* 2016a) on date palm trees showed no significant differences in the palm leaves content of chlorophyll pigments (a l b) when treating the soil at a concentration of (10 $mg.kg^{-1}$) of cadmium from control treatment while adding cadmium at a concentration of (30 $mg.kg^{-1}$) to the soil reduced the chlorophyll pigments, with a significant difference and a percentage of 17% for chlorophyll (a) and 39% for chlorophyll (b) compared to the control treatment. Al-Jabri, (2017) also found that treating date palm trees (Barhi cultivar) with cadmium at a concentration (9 $mg.kg^{-1}$)

had a significant effect in raising the leaves content of total phenolic compounds from (5.03 mg.g^{-1} dry weight) in the control treatment to (8.63 mg.g^{-1} dry weight), with a significant difference from the rest of the treatments except the lead treatment (276 mg.kg^{-1}). The present study was conducted to determine the effect of pollution with heavy elements resulting from fuel combustion from oil wells on the pigment of total chlorophyll and some chemical traits of date palm leaves (Hilawi cultivar).

Materials and Methods

This study was conducted in five private orchards, four of which belong to Al-Deer district which is located in each of the regions (Nahr Bin Omar, Al-Zwain, Al-Jarahi and Um Masjid) and the fifth is located in Al-Saraji region belonging to Abu Al-Khaseeb District as a control treatment. The locations were with different dimensions from the source of pollution (South Gas Company, Production Department, Bin Omar Production Division) as shown in table 1. The experiment was conducted during the period 25/2/2018 until 9/30/2018, where three replicates were selected (palm trees) for each location of date palm trees (Hilawi cultivar) that similar as possible in age and vegetative growth, planted in lines with dimensions of (8×8 m) and irrigated by the running water from the Shatt al-Arab River. The soil analysis process was conducted for the study locations by taking three samples from each location with a depth of (0 - 40) cm and a distance far (1-2 m) from the stem of the palm in the orchard and air-dried for 72 hours and gravel and impurities were removed and then crushed and sieved with a sieve of (600 µm). It was then transferred to the Laboratory of Marine Sciences Center and the Central Laboratory, College of Agriculture, Basra University to perform some laboratory analyzes as shown in table 2 and Fig. 1, in addition to estimating the concentrations of heavy metals as shown in table 3. The total chlorophyll pigment in the leaves was estimated according to the Holden method described by (Howertiz, 1975) using the Spectrophotometer. The total chlorophyll pigment was calculated according to the following formula:

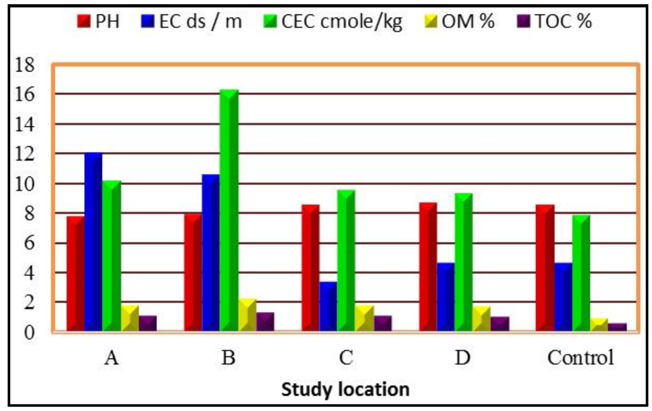

Fig. 1: Some physical properties for the soil of study locations with different dimensions from the source of pollution.

Table 2: Soil texture and volumetric distribution for the soil of study locations.

Trait	Unit	Control	A	B	C	D
Sand	%	39	17	18.02	51.3	38.6
Clay	%	32	25	32.9	15.5	21.7
Silt	%	29	58	49.08	33.2	39.7
Soil texture		Clay loam	Silt Loam	Silty Clay loam	Silt	Silt

Table 3: The total concentration of heavy metals (mg.kg^{-1}) in the soil for the study locations.

Element Location	Lead	Cad-mium	Iron	Zinc
Control	26.326	1.263	5297.809	74.651
A	73.504	6.231	4139.237	284.029
B	49.524	5.194	5052.972	229.113
C	38.968	3.101	5324.450	226.428
D	29.584	2.067	5456.891	158.697
L.S.D p ≤0.05	8.81	1.318	761.7	40.30

Total chlorophyll (mg.L^{-1}) = 20.2 * OD (645) + 8.02 * OD (663)

For the purpose of converting the total amount of chlorophyll dye from (mg.L^{-1}) to (mg. 100 g^{-1} fresh weight) we use the following formula:

mg. 100 g^{-1} fresh weight =

$$\frac{mg.L^{-1}}{1000\,ml} \times \frac{100}{sample\ weight\ (g)}$$

Table 1: The different study locations and the distance from the source of pollution and the administrative location of Basra province.

Location	Distance from pollution source (m)	The administrative location in Basra province	The symbol for the study location
The first location	207	Nahr bin Omar - Al-Deer District	A
The second location	2500	Al Zuwayn - Al-Deer District	B
The third location	4000	Al-Jarahi - Al-Deer District	C
Fourth location	7000	Um Masjid - Al-Deer District	D
Control treatment	35000	Al-Saraji - Abu Al-Khaseeb District	Control

where

OD = Optical Density Reading

OD (645) = optical absorption reading at 645 nm wavelength

OD (663) = optical absorption reading at 663 nm wavelength

The leaves content of total soluble carbohydrates was determined according to the phenol-sulfuric acid method based on (Dobois *et al.,* 1956). Phenolic substances were also estimated according to the Folin-Denis method mentioned in (Dallali and Al-Hakim, 1987). As for the proline amino acid, it was estimated according to the method (Bates *et al.,* 1973) and described by (Hussain *et al.,* 2011). The experiment was designed using a Randomized Complete Block Design (RCBD), with one-factor, the results were then analyzed using a one-way ANOVA variance analysis l using the ready-made statistical program (Genstat, 2013) to analyze the data of the studied traits and the differences between the averages were compared using the Least Significant Difference Test (LSD), with a probability level of 0.05.

Results and Discussion

The effect of study locations with different dimensions from the source of pollution on the total amount of total chlorophyll pigment in the date palm leaves (Hilawi cultivar)

The results of the data showed a significant decrease in the concentration of total chlorophyll pigment in the locations (A, B, C) compared to the Control location which amounted to (2.69, 3.37 and 3.79 mg. 100 g^{-1}), respectively, While no significant differences were observed between the two locations (D and Control) which amounted to (4.25 and 4.87 mg. 100 g^{-1}), respectively as shown in Fig. 2. This decrease in the total chlorophyll pigment can be attributed to the fact that heavy elements influence the process of Chlorophyll Biosynthesis due to inhibition of the necessary enzymes in this process such as dehydratase ä-aminolevulinic acid and porphobilinogen deaminase responsible for the formation of Porphyrin which is the main component of plant pigments (Prasad and Prasad, 1987; Parmar *et al.,* 2013; Elloumi *et al.,* 2014). In addition, the cadmium element replaces the central magnesium (Mg) atom in the Chlorophyll molecule forming (Cd complex-Chlorophyll). This complex may cause a deterioration in the function of photosynthesis which may lead to plant death. The cadmium element also works to compete with the iron element that is associated with the photosynthesis cytochrome and competing for the manganese element

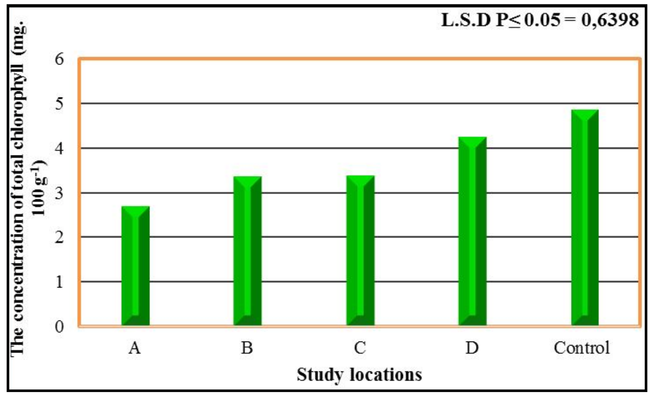

Fig. 2: Effect of study locations with different dimensions from the source of contamination on the amount of total chlorophyll pigment in date palm leaves ((Hilawi cultivar)) (mg. 100 g^{-1}).

that contributes to the oxygen release reactions, and its substitution in place of these two elements leads in turn to the processes of photosynthesis and respiration (Hart and Scaife, 1977). In addition, the low content of chlorophyll has to do with the increase of reactive oxygen species (ROS) that stimulate the second photovoltaic system and lead to a change in chlorophyll (Gomes *et al.,* 2015). The decrease in the total chlorophyll pigment can also be attributed to the heavy elements such as lead and cadmium table 3 affected the activity of metabolic activities, causing a change in the levels of photosynthetic pigments. The lead element also distorts the structure of the chloroplast membrane, which leads to a decrease in the chlorophyll content (Bhardwaj *et al.,* 2009). Heavy elements are known to interfere directly to inhibit the action of some enzymes necessary for chlorophyll synthesis and photosynthesis, or by stimulating a lack of essential nutrients (Van Assche and Clijsters, 1990; Meers *et al.,* 2010). Moreover, the destruction of chlorophyll was associated with the stress of heavy elements in several plants (Cozzolino *et al.,* 2010; Gupta *et al.,* 2013). This result is identical to that obtained by numerous studies that showed that the decrease in total chlorophyll pigment in plant leaves is due to exposure to heavy elements stress (Bhardwaj *et al.,* 2009; Doganlar *et al.,* 2012; Vineeth *et al.,* 2015; Bharti *et al.,* 2017).

The effect of the study locations with different dimensions from the source of pollution on the total amount of total soluble carbohydrates in the date palm leaves (Hilawi cultivar).

It is noted from the data that there are significant differences between the locations in this trait, where the location (A) was significantly excelled on the rest of the locations, which did not differ significantly from the location (B) which amounted to (24.17 and 22.98 mg.g^{-1} dry weight), respectively. As for the control location, it recorded the lowest amount of total soluble carbohydrates, which amounted to (18.06 mg.g^{-1} dry weight) as shown in Fig. 3. Perhaps the reason for the high concentration of soluble sugars in plants exposed to the stress of heavy elements is due to starch degradation to meet energy production requirements for physiological activity, where A positive relationship has been observed between the accumulation of soluble sugars with plants tolerating mineral stress (Karimi *et al.,* 2012). Or, the reason for the increase in the accumulation of carbohydrates in plants subject to mineral stress may be due to the inhibition of one or more of the three main steps in the process of transferring carbohydrates from the source (leaves), which causes a decrease in the use of carbohydrates for growth although the low growth and the accumulation of high carbohydrates in The plant cannot always be connected (Samarakoon and Rauser, 1979; Greger *et al.,* 1991). These results agree with the results of many studies that have shown that plants prone to mineral stress tend to accumulate carbohydrates in their tissues (Balsberg-Pahlsson, 1998; Zouari *et al.,* 2016; Jian *et al.,* 2017; Al-Jabri, 2017). it also agrees with (Zouari *et al.,* 2016 b) who found when treating with cadmium led to an increase in the concentration of soluble sugars in date palm leaves in contrast to decreased starch content, because date palm tends to increase the construction and development of antioxidant defense systems in response to the harmful effects of cadmium.

The effect of the study locations with different dimensions from the source of pollution on the amount of total phenols in the dates palm trees (Hilawi cultivar).

It is noted from the data that there are significant differences between the locations in this trait, where the location (A) was significantly excelled on the rest of the locations, which did not differ significantly from the location (B) which amounted to (12.13 and 10.94 mg.g^{-1} dry weight), respectively, While the control location gave the lowest amount of total phenols, which amounted to (6.69 mg.g^{-1} dry weight) as shown in Fig. 4. This may be due to the presence of phenolic compounds in plants having multiple biological effects as antioxidant activity (Wojdylo *et al.,* 2007). When plants are exposed to heavy elements stress, ROS production increases, and ROS production in plant cells is balanced by antioxidant compounds and enzymes such as ascorbic acid, glutathione, and phenolic compounds that inhibit oxidation occurrence and have a critical role in stress responses (Racchi, 2013). In high-end plants, phenolic compounds are considered to be secondary metabolites and have roles that contribute to responding to environmental stresses where they participate in many physiological processes associated with plant growth and development (Tattini *et al.,* 2004; Garica-Sanchez *et al.,* 2012). The increase in phenolic compounds exposed to the stress of heavy elements can be explained by the increase in the production of enzymes involved in metabolism, which in turn contributes to building phenolic compounds in the leaves which are strong alternative antioxidants such as flavonoids, tannins, and Quinine that act as a transporter of metal ions and inhibit ROS compounds thus leads to the prevention or inhibition of lipid oxidation (Sharma *et al.,* 2012; Zouari *et al.,* 2016). This result agrees with the results of studies by many researchers (Loponen *et al.,* 1998; Furlan *et al.,* 1999; Robles *et al.,* 2003; Jiang *et al.,* 2017).

The effect of the study locations with different dimensions from the source of pollution on the amount of the amino acid (proline) in the date palm leaves (Hilawi cultivar).

The data showed that there are significant differences

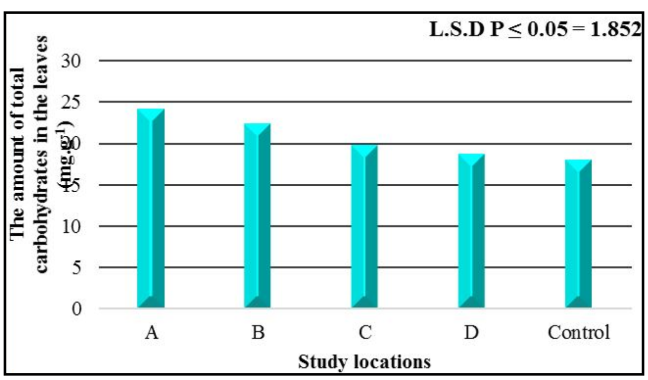

Fig. 3: Effect of study locations with different dimensions from the source of pollution on the amount of total soluble carbohydrates in the date palm leaves (Hilawi cultivar) (mg.g^{-1}).

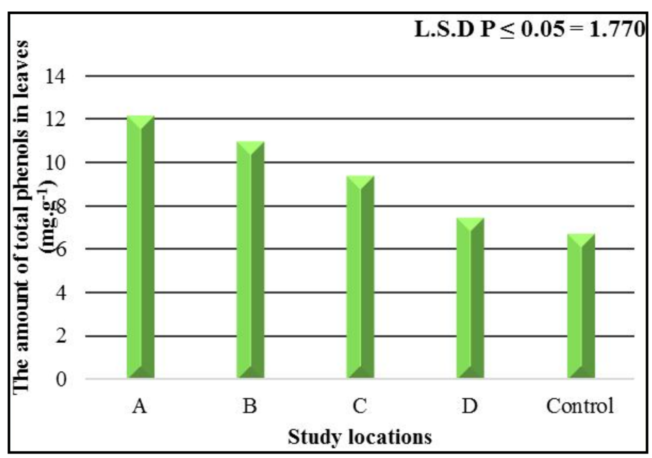

Fig. 4: Effect of study locations with different dimensions from the source of pollution on the amount of total phenols in the date palm leaves (Hilawi cultivar) (mg.g^{-1}).

between the locations in this trait, where the location (A) was significantly excelled on the rest of the locations, which did not differ significantly from the location (B) which amounted to (13.01, 11.78 µmol.g^{-1} fresh weight), respectively while the Control location recorded the lowest amount of proline amounted to (7.00 mol.g^{-1} fresh weight) as shown in Fig. 5. Perhaps the reason for this is that the proline accumulation is a strategy of adaptation for plants in the environment exposed to any stresses by maintaining the osmotic balance, removing and inhibiting the formation of free radicals, maintaining the stability of the cell membrane, and has a protective role in the oxidation of fats and the maintenance of the second photovoltaic system and the process of transport Electron (Ashraf and Foolad, 2007; Ben Ahmed *et al.,* 2010; Asgher *et al.,* 2013; Dawood *et al.,* 2014; Singh *et al.,* 2015). The accumulation of free proline in response to the stress of heavy metals appears to be a common adaptation in plants, since adding proline to growing plants under the stress of heavy elements does not only reduce the absorption of plant roots to the elements but also enhances their exclusion, and therefore the external proline can form a barrier against the absorption of Heavy elements (Islam *et al.,* 2009). Tahri *et al.,* (1997) also showed an inverse relationship between the level of proline accumulation and a decrease in the content of total chlorophyll pigment, Where Ledily *et al.,* 91993) concluded that gabaculine is the common initiating compound for the synthesis of both chlorophyll and proline, and therefore competition between them occurs. This emphasizes the results in this study, where a decrease in the concentration of chlorophyll pigment was observed in palm trees growing in locations contaminated with heavy elements while increasing the content of their leaves from the free amino acid proline. These results agree with the results of many studies that showed that the plant's exposure to heavy metal stress led to an increase in the accumulation of the protein amino acid in its leaves (Oncel *et al.,* 2000; Bandehagh, 2013; Nareshkumar *et al.,* 2015; Jiang *et al.,* 2017).

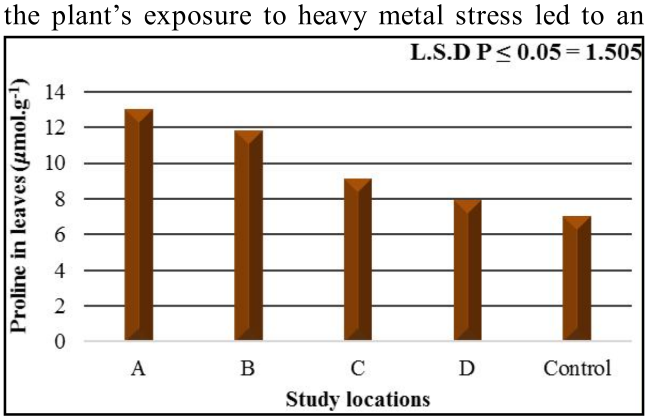

Fig. 5: Effect of study locations with different dimensions from the source of pollution on the amount of the amino acid (proline) in the date palm leaves (Hilawi cultivar) (mg.g^{-1}).

According to the above, we conclude that far or near the distance between the date palm trees (Hilawi cultivar) and the source of pollution (South Gas Company, Production Department, Bin Omar Production Division) has a direct impact on the traits studied above.

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