Alzurfi et al. *Iraqi Journal of Science, 2019, Vol.60, No.10, pp: 2141-2148*
DOI: 10.24996/ijs.2019.60.10.6

ISSN: 0067-2904

Effect Different Concentrations of Crude Oil on the Pigment Content and Protein Content of *Hydrilla Verticillata* Plant

Sadiq Kadhum Lafta Alzurfi*, Kasim kadhim alasedi, Noor Imad Abdulraheem
Department of Ecology, Faculty of Science, University of Kufa, ALnajaf, Iraq

Abstract

The current study was conducted to determine the effect of crude oil on the pigments and protein content of *Hydrilla verticillata* plant from December 2018 to February 2019. *Hydrilla verticillata* was exposed to different concentrations of treatments (0.01,0.05,0.10,0.20) % of crude oil for 24 days. The pigments content was evaluated as total chlorophyll, a, b, carotene and protein content within plant tissues during the days (1, 8, 16 and 24).

The results of this study showed an increment in the total chlorophyll values of plant, which is exposed to different concentrations of crude oil compared to the control treatment during the first day of the experiment and decreased compared to control treatment during 8,16 and 24 days of the experiment. The recorded maximum value of total chlorophyll was (29.49) µg / g at 0.10% treatment during the first day and the statistical analysis showed significant differences below the probability level (P> 0.05) between the concentrations and the days of the experiment. Chlorophyll a, b and carotene of plant exhibit the overall trend of total chlorophyll. The values of chlorophyll b were higher than the values of chlorophyll and carotene.

The current study showed a gradual decrease in the total protein values of plants in crude oil during experiential period compared to control treatment, recording the highest values at the 0.01% during the first day (29.4 mg/g) and the lowest in the concentration of 0.05% during the day 24 was (3.5) mg / g, and the statistical analysis showed significant differences below the probability level (P> 0.05) between the days and concentrations of the experiment. The study concluded that a gradual decrease of total chlorophyll values during period experimental, recorded the chlorophyll b values higher compare to chlorophyll a and carotene. the gradual decrease of protein values with the increasing the concentration.

Kewwords: Crude,Concentrations, Hydrilla verticillata, Oil, Protein.

تأثير تراكيز مختلفة من النفط الخام على محتوى الصبغات والمحتوى البروتيني لنبات *Hydrilla verticillata*

صادق كاظم لفته الزرفي*، قاسم كاظم محمد الأسدي، نور عماد عبد الرحيم
قسم علم البيئة، كلية العلوم ، جامعة الكوفة، النجف، العراق

الخلاصة

أجريت الدراسة الحالية لتحديد تأثير النفط الخام على أصباغ ومحتوى البروتين في نبات الهايدريلا *Hydrilla verticillata* من كانون الاول 2018 إلى شباط 2019. تم تعريض *Hydrilla verticillata* لتراكيز مختلفة من المعاملات (0.01،0.05،0.10،0.20) ٪ من النفط الخام لمدة 24 يوما. تم تقييم محتوى

*Email: Sadiqk.alzurfi@uokufa.edu.iq

الكلوروفيل على أنه محتوى الكلوروفيل الكلي، a، b، الكاروتين والبروتين داخل الأنسجة النباتية خلال الأيام (1، 8، 16 و24). أظهرت نتائج هذه الدراسة زيادة في إجمالي قيم الكلوروفيل للنبات، والتي تتعرض لتركيزات مختلفة من النفط الخام مقارنة بمعاملة السيطرة خلال اليوم الأول من التجربة وانخفضت مقارنة بمعاملة السيطرة خلال اليوم (8, 16 و 24) من التجربة. سجلت اعلى قيمة للكلوروفيل الكلي (29.49) ميكروغرام / غرام عند معاملة 0.10 ٪ خلال اليوم الأول وأظهر التحليل الإحصائي فروق معنوية ذات دلالة إحصائية تحت مستوى احتمالية (P> 0.05) بين التراكيز وأيام التجربة. اخذت قيم الكلوروفيل أ، ب والكاروتينات للنبات ميلا بالاتجاه العام للكلوروفيل الكلي. كانت قيم الكلوروفيل ب أعلى من قيم الكلوروفيل أ والكاروتينات.

أظهرت الدراسة الحالية انخفاضًا تدريجيًا في إجمالي قيم البروتين للنبات خلال الفترة التجريبية مقارنةً بمعاملة السيطرة، حيث سجلت أعلى القيم عند 0.01٪ خلال اليوم الأول (29.4 ملغم / غم) وأدنى معدل سجل في تركيز 0.05 ٪ خلال اليوم 24 كانت (3.5) ملغم / غم، وأظهر التحليل الإحصائي فروق معنوية ذات دلالة إحصائية تحت مستوى احتمالية (P>0.05) بين تراكيز وأيام التجربة. واستنتج من الدراسة انخفاض تدريجي في قيم الكلوروفيل الكلي خلال الفترة التجريبية، سجلت قيم الكلوروفيل ب أعلى مقارنة بالكلوروفيل أ والكاروتينات. وانخفاض تدريجي في قيم البروتين مع زيادة التركيز.

Introduction

Oil pollution is a common problem worldwide. The problem of oil pollution has emerged because of the rapid technological development in the oil industry, which resulted in an increase in its production and increase in the irregular use, so increased the introduction of oil and its derivatives to the environment, especially the water environment for many transportation accidents [1],and crude oil is a complex mixture of thousands of hydrocarbons and nonhydrocarbon compounds, Total petroleum hydrocarbon (TPH) is a term used to describe a large family of several hundred chemical compounds that originally come from crude oil. Crude oil is used to make petroleum products, which can contaminate the environment [2].

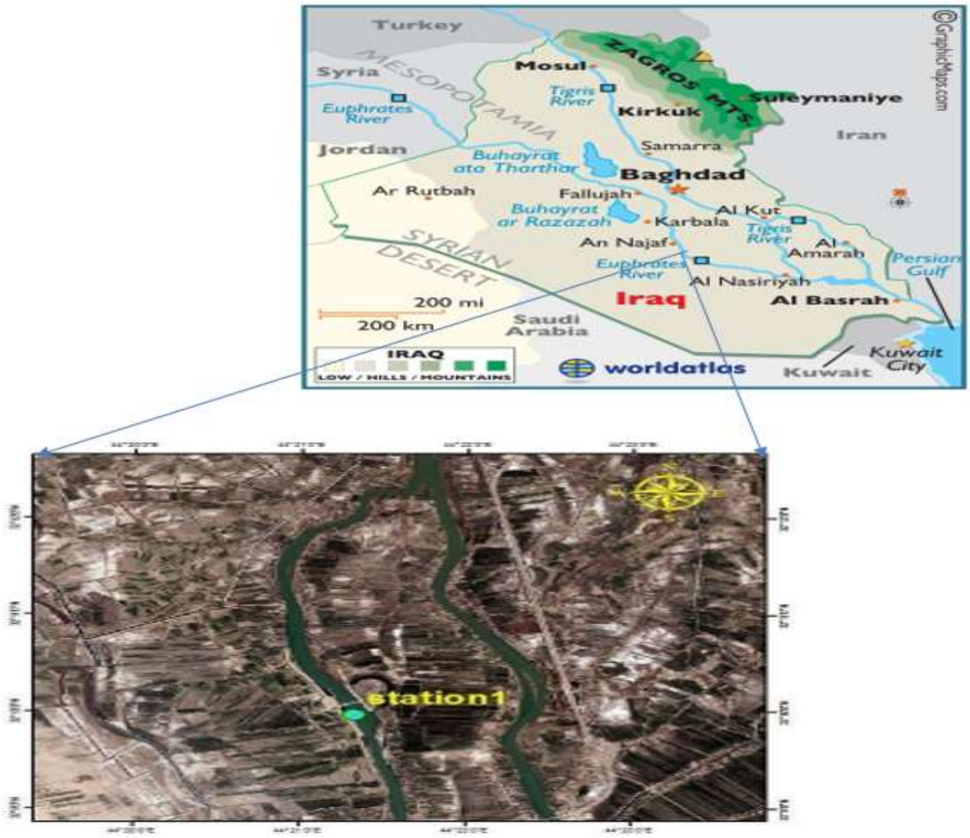
TPH is a mixture of chemicals, but they are all made mainly from hydrogen and carbon, called hydrocarbons. Some chemicals that may be found in TPH are hexane, jet fuels, mineral oils, benzene, toluene, xylenes, and naphthalene, as well as other petroleum products and gasoline components. However, it is likely that samples of TPH will contain only some, or a mixture, of these chemicals [2]. The chemical composition of crude oil varies significantly and can have diverse effects on different organisms within the same ecosystem.[3] .These differences in toxic effects are due to qualitative compositional differences in the various products, as well as concentration differences of the chemical constituents[4,5] Researches showed that Hydrocarbons compounds accumulated in the plants cells suppressed their photosynthesis by reducing the primary photochemical yield, increase of energy expenditures [6] Oil also could block the absorption of CO2 and nutrients, leading to decrease chlorophyll a and reduce of primary productivity. Oil would destroy the cell structure and membrane system of plants and disturb the operation of anti-oxidation defense system, stop the synthesis of nucleic acid and protein, even induce the cell abnormality and gene mutation [7].

The use of aquatic macrophytes for treatment of oil pollution to mitigate a variety of pollution level is one of the most researched issues all over the world. Phytoremediation is green technologies that use plants to remove contaminant from the environment. As well as is technology environmentally friendly and potentially cost- effective. Phytoremediation classified in containment, degradation, extraction pollutant. Sometimes combination of these methods used for remediation. Plant could contaminant from the water and concentrate them in their tissues, and degraded pollutant by biotic and abiotic process. Plants could uptake solvent, aromatic hydrocarbons and other organic contaminant from the water, underground water. Mechanism involves in Phytoremediation process are Phytodegradation Phytoextraction, Phytoaccumulation, Rhizodegradation and Phytovolatilization.

The selection of the aquatic plant species is one of the skilled tasks prior to the design of a water treatment facility. An effort has been made in this review to cover the most researched aquatic flora for mitigation purposes and their possible use in treatment as the selection of an appropriate aquatic plant species reduce the time and cost of the treatment processes. Recent many of researcher investigation on *H. verticillata* plant about impossibility this plant to tolerate any pollutant such as heavy metal, pesticides, and hydrocarbons. [8-11]. The study aimed to study the effect of hydrocarbons on the pigments content and protein of *Hydrilla verticillata*.

Material and Methods:

Plant samples of *H. verticillata* were collected from the Euphrates River (Al-Zarqa region in Kufa city) located between Longitude °32 99´ 861´´N and Latitude °44 28´ 10´´E.(Map 1). The experiment was carried out in the culture room that included constant temperature (28 C̊) and light (500 lux) during the period from December 2018 to February (2019) at the college of science, University of Kufa(Najaf, Iraq).



Map 1-Map represented the collecting position of the samples from Euphrates River

Experimental Design

Terminal shoots of both species were cut into 15 cm length fragments while existing branches, roots and flower buds were removed. They were planted in plastic containers all were equal dimensions (40cm length x 25cm width x 25cm height) filled with 10 liters of water per container and the water level of each container was maintained at a constant level throughout the experiment. Our experiment was designed in 15containers (plastic tank) divided into five treatments, for each treatment with three replications. In the four treatments contain different concentrations of crude oil, a fifth treatment were only planted without crude oil (control).

The experimental was arranged in Completely Randomized Design and plant growth parameters (Chlorophyll content and protein content) were recorded after (1, 8, 16 and 24) day of planting after acclimatized of the plant for 14 days in tap water; after acclimation, plant exposed to chosen concentrations of the crude oil; (0.01%, 0.05%,0.10% and 0.20%), with constant light irrationally (500 Lux.); photoperiod (12/12 light/dark) and temperature 28C̊.

Plant growth parameters measured total chlorophyll, chlorophyll a and chlorophyll b according to [12] by spectroscopy type (Spectrophotometer SP-300) at waves (470,645 and 663) nm.

Used the Biuret method to estimate the protein in plant tissues, were measured Absorbance by Spectrophotometer(SP-300) at a 555 nm wavelength. Bovine protein solution was used in the preparation of the standard curve and the protein content was expressed in mg / g plant tissue [13].

Statistical analysis

The experimental plots were arranged in Completely Randomized Design;data were analyzed by using SPSS statistical software (version 16)

Results and Discussion:

Chlorophyll is the green pigment responsible for photosynthesis (energy production) and is found inside the plant cell in the plastids [14]. The chlorophyll content of the plant cell is affected by the presence of essential elements such as calcium and magnesium that responsible for chlorophyll formation. Many elements are necessary for the plants' growth and the formation of chlorophyll pigment that is responsible for the process of photosynthesis, including iron, as it has a key role in the formation of chlorophyll through the presence of many of the coenzymes, including Cytochrome Oxidase and Catalase and Peroxidase [15].

The current results showed a decrease in the ratio of total chlorophyll of plants under study, the total chlorophyll was recorded with a maximum value 29.5 µg/g in (0.10 %) treatment of crude oil during 1 day and the minimum value was 6.04 µg/g in (0.20%) during 24th days of experimental Statically analysis under probability ($p<0.05$) showed there are significant differences between all interactions (Figure-1). The mean of chlorophyll a of plants in the current study was recorded with a maximum value recorded in (0.05%) treatment of *H. verticillata* was 6.08 µg/g during 1 day and the minimum value recorded in (0.10%)treatment was 1.13 µg/g during 16th days (Figure-2).While chlorophyll b was recorded high values of *H. verticillata* in (0.05%) treatment was 24.1 µg/g during 1 day and minimum value was 4.84µg/g in0.20%treatment during 24th days of experimental (Figure-3);The mean of plant carotene in the current study was recorded with a maximum value recorded in (0.10%) treatment was 2.94 µg/g during 1 day and the minimum value recorded in (0.01%) treatment was 0.82 µg/g during 8 days of experimental (Figures-4, 5). Statistical analysis under probability ($p<0.05$) showed there are significant differences between all interactions.

The existence of crude oil in the water may be a reason to inhibit the synthesis of chlorophyll in *Hydrilla verticillata* plant. Crude oil is a combination of aliphatic and aromatic molecules and high molecular weights of organic compounds that inhibit the enzymes needed to chlorophyll synthesis [16]. [17] explained with a high concentration of crude oil decreases the total chlorophyll content, chlorophyll a, chlorophyll b, and carotenoids significantly decrease. The low chlorophyll content in the plant was an indicator of environmental pollution [17]. [18] showed that the decrease in the total chlorophyll content in the leaves may be due to the alkaline state resulting from the solubility of the chemicals in the oil into the cell extractor responsible for degradation of chlorophyll. Previous studies have shown that total chlorophyll content decreases in oil-contaminated leaves compared to control treatment [19,20].This is also due to the fact that the crude oil in the water causes a "visible" change in the color of the water and thus affects the transparency of the water by reducing the passage of light and thereby inhibiting the photosynthesis process in chloroplasts as well as the formation of ROS (Reactive Oxygen species) Plant that directly or indirectly affects photosynthesis [21].The sensitivity of chlorophyll a and b during day 7 and explains the effect of the Photosystem II on complex external factor [22]

Studies have shown that low chlorophyll b content will affect the stability of the photosystem, resulting in unstable photosynthesis and reduced plant tolerance to stress. Conversely, chlorophyll b content may increase under pressure, possibly due to increased plant tolerance, and the plant can adapt to Difficult conditions by controlling the chlorophyll ratio (a and b) [23]. The inhibition of photosynthesis leads to increased deterioration of chlorophyll pigments to produce carbon to meet the energy needs of cells. It is also suggested that when photosynthesis is inhibited, cells speed up the process of dye decomposition to maintain the balance between energy use and absorption [24]

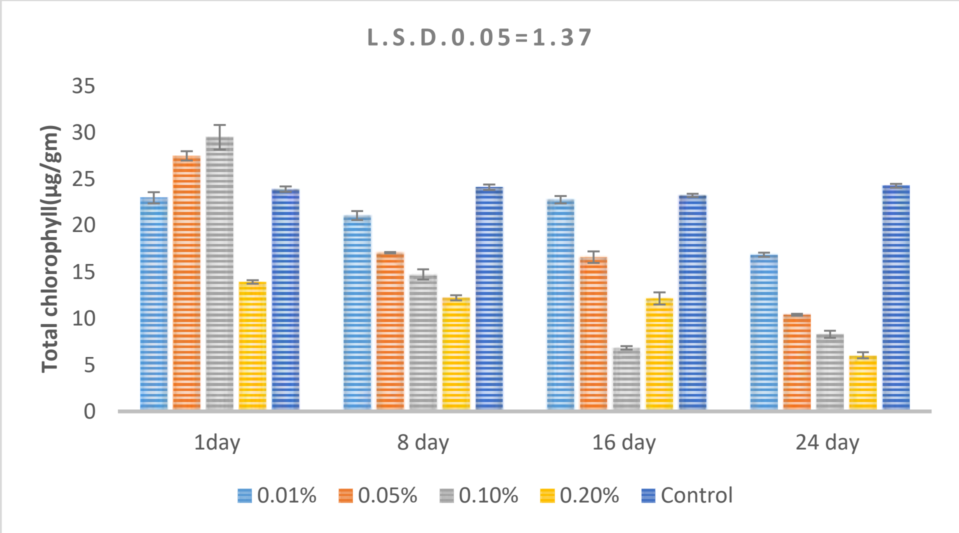


Figure 1-Means total chlorophyll of *H. verticillata* after exposed to different concentration of crude oil during the experimental period.

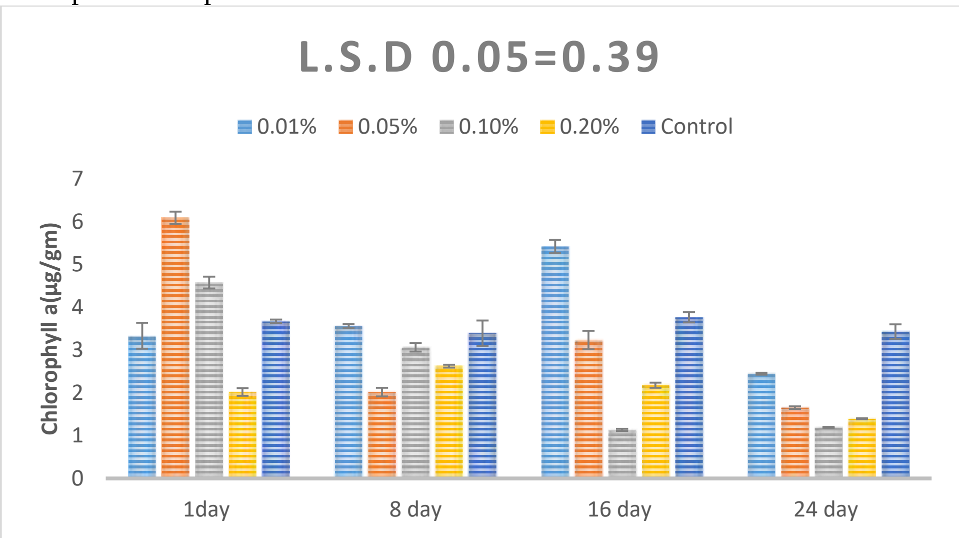


Figure 2-Means chlorophyll a of *H. verticillata* after exposed to different concentration of crude oil during the experimental period.

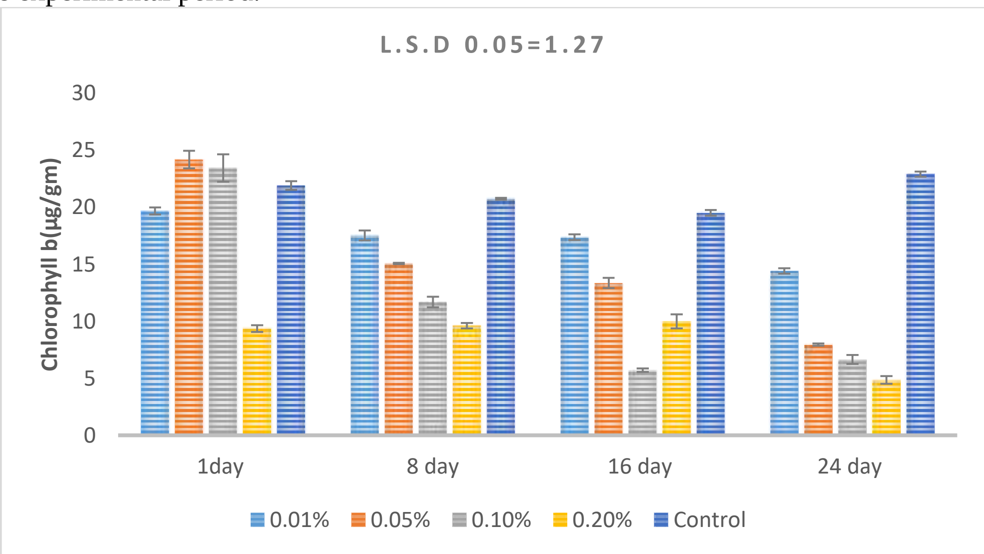


Figure 3-Means chlorophyll b of *H. verticillata* after exposed to different concentration of crude oil during the experimental period.

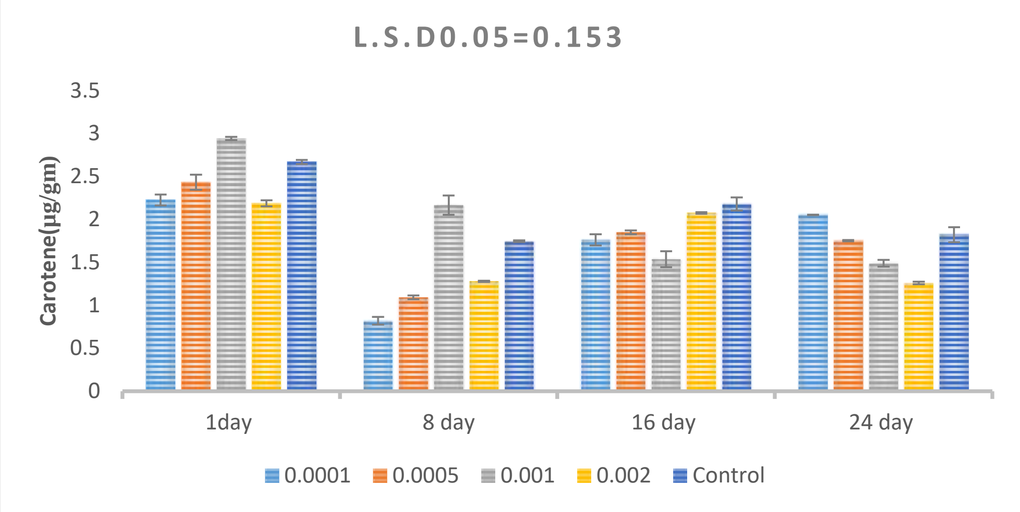


Figure 4-Means carotene of *H. verticillata* after exposed to different concentration of crude oil during the experimental period.

Protein plays an "important" role in the metabolism and plasma membrane, where it regulates the processes that overlap the external and internal membrane [25]. Dissolved protein content is an "important" indicator of the plant's physiological state [26] The results of the study showed a decrease in protein content in plants during the treatment period due to plant stress caused by ROS (Reactive Oxygen Species), which are oxygen-reactive chemical molecules such as Superoxide anion (O_2), hydrogen peroxide (H_2O_2) Hydroxyl (OH-). Leading to oxidative stress that produces these compounds as transverse products during metabolism that affect plant cells and lead to their death, as well as the breakdown of protein, fat, and DNA [27].Studies have shown that plants can slow down the rate of protein synthesis when under stress conditions, thereby reducing protein content [28].

After the exposure of the plant to crude oil for 16 days, the differences in protein content in *H. verticillata* were not important because the plant antioxidant enzymes were activated, and the stability of the organism was maintained. However, with the increased concentration of crude oil, the protein content gradually decreased. May be due to the breakdown of soluble proteins in the exposed plant or due to the increased activity of protease or other proteolytic enzymes that activated and destroyed the protein[29].

Conclusion:

H. verticillata has been affected when exposed to high concentration of hydrocarbons via chlorophyll content and protein content and will have the ability to tolerate hydrocarbons at low concentrations.

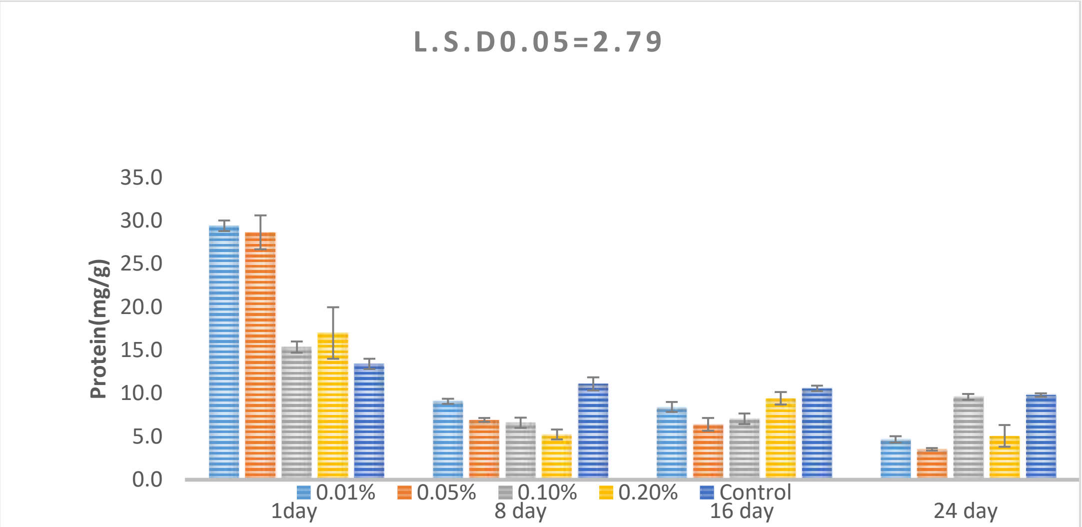


Figure 5-Means total protein of *H. verticillata* after exposed to different concentration of crude oil during the experimental period.

References:

1. Kvenvolden K.A.and Cooper C.K.**2003.** Natural seepage of crude oil into the marine environment. *Geo-Marine Letters*, 23, 3-4, pp 140–146.
2. Agency for Toxic Substances and Disease Registry (ATSDR). **1999.** Toxicological profile for total Petroleum hydrocarbon. Atlanta, GA: U.S. Department of Health and Human Services, Public Health.Service.
3. Lübcke-von Varel U, Machala M, Ciganek M, Neca J, Pencikova K, Palkova L, et al. 2011. Polar compounds dominate in vitro effects of sediment extracts. *Environ Sci Technol*.; **45**(6): 2384–90. doi: 10.1021/ es103381y PMID: 21348526**.**
4. Albers, P. H. 1995. Petroleum and individual polycyclic aromatichydrocarbons. *Handbook of ecotoxicology*, *2*, 330-355. Sverdrup, L.E.; Krogh, P.H.; Nielsen, T.; Kjور, C.; Stenersen, J. Toxicity of eight polycyclic aromatic compounds to red clover (Trifolium pretense), ryegrass (Lolium perenne), and mustard (Sinapsis alba). Chemosphere 2003, 53, 993–1003**.**
5. Sverdrup,L.E;Krogh,P.H;Nielsen,T;Kjr,C;Stenersen,J.2003.Toxicity of eight polycyclic arimatic compounds to red clover(*Trifolium pretense*).ryegrass(*Lolium perenne*),and mustard(*Sinapiss alba*).Chemosphere,53,993-1003.
6. Aksmann, A. and Tukaj, Z. **2008.** Intact anthracene inhibits photosynthesis in algal cells: a fluorescence induction study on Chlamydomonas reinhardtii cw92 strain. *Chemosphere*, **74**(1): 26-32**.**
7. Bopp, S. K. and Lettieri, T. **2007.** Gene regulation in the marine diatom Thalassiosira pseudonana upon exposure to polycyclic aromatic hydrocarbons (PAHs). *Gene*, **396**(2): 293-302
8. Alwan, S. W. **2015**. The Bioaccumulation and Toxic Effect of Pyrene and Phenanthrene in Hydrilla verticillata (LF) Royal. *Journal of Kerbala University*, **13**(4): 113-121.
9. Abdul-Alghaffar, H. N. and Al-Dhamin, A. S. **2016.** Phytoremediation of chromium and Copper from aqueous solutions using Hydrilla verticillata. *Iraqi Journal of Science*, **57**(1A): 78-86**.**
10. Al-Zurfi, S. K. L., Alisaw, A. Y. and Al-Shafai, G. A. A. **2018.** Anatomical and Physiological Effectsof Cadmium in Aquatic Plant*Hydrilla verticillata*. *Plant Archives*, **18**(1): 839-846.
11. Al-Zurfi, S. K. L., Albdari, N. H. and Al-Shamari, F. R. A. **2019**. Impact of Glyophosate and Lambda Cyhalothrin Pesticides on Physiological and AnatomicalVariations Of The *Hydrilla verticillata*.Plant *Plant Archives*, **19**(1): 839-846.
12. Arnon DI. **1949.** copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris. *Plant physiology*, **24**: 1-15.
13. Pak, J. **2010.** Analysis of portion by spectrophotometric and computer colour based intensity method form stem of pea (Pisum sativum) at different stages . *Anal. Environ. Chem*. **11**(2): 63-71.
14. Lefsrud, M. G., Kopsell, D. A., Kopsell, D. E. and Randle, W. M. **2006.** Kale carotenoids are unaffected by, whereas biomass production, elemental concentrations, and selenium accumulation respond to, changes in selenium fertility. Journal of agricultural and food chemistry, **54**(5): 1764-1771.
15. Sarvari, E., Cseh, E., Blczer, T., Szigeti, Z., Zaray, G. and Fodor, F. **2008.** Effects of Cd on the iron re-supply induced formation of chlorophyll – protein complexes in cucumber. *Acta Biologica Szegediensis*, **52**(1): 183 – 186.
16. Anthony, P. **2001**. Dust from walking tracks: impacts on rainforest leaves and epiphylls. Cooperative Research Centre for Tropical Rainforest Ecology and Management, Australia. American Society for test andmaterials-ASTM D3921 and D5369.
17. Al-Hawas GHS, Sukry WM, Azzoz MM, Al-Moaik RMS **2012**. The effect of sublethal concentrations of crude oil on the metabolism of Jojoba (Simmodsia chinensis) seedlings. Int Res J Plant Sci, **3**(4): 54–62.
18. Njoku, KL., Akinola, MO. and Busari, TO. **2012** Effect of time of application of spent oil on the growth and performance ofmaize (Zeamays). *Afr J Environ Sci Technol*, **6**: 67–71
19. Mandal, M. and Mukherji, S. **2000.** Changes in chlorophyll context, chlorophyllase activity, hill reaction, photosynthetic CO uptake, sugar and starch content in five dicotyledonous plants exposed to automobile exhaust pollution. J Environ Biol, **21**: 37–41.

20. Odjegba ,V.J. and OKUNNU, O. O. **2012.** Effects of simulated crude oil pollution on the growth of Manihot esculenta Crantz. *Indian Journal of Science*, **1**(2): 116-119.

21. Liu, H., Weisman, D., Ye, Y B., Cui, B., Huang, Y H., Colon-Carmona, A. and Wang, Z H. **2009.** An oxidative stress response to polycyclic aromatic hydrocarbon exposure is rapid and complex in Arabidopsis thaliana. *Plant Science*, **176**(3): 375--382.

22. Romero, M., Martin-Cuadrado, A. B., Roca-Rivada, A., Cabello, A. M. and Otero, A. **2011.** Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. *FEMS Microbiol. Ecol*. **75**: 205–217. doi: 10.1111/j.1574-6941.2010.01011.x.

23. JIANG B., CHEN J., LUO Q., LAI J., XU H., WANG Y., YU K. **2016.** Long-term changes in water quality and eutrophication of China's Liujiang River. *Pol. J. Environ. Stud*. **25**: 1033.

24. Mohapatra, P. K., Patra, S., Samantaray, P. K. and Mohanty, R. C. **2003.** Effect of the pyrethroid insecticide cypermethrin on photosynthetic pigments of the cyanobacterium Anabaena doliolum Bhar. *Polish Journal of Environmental Studies*, **12**(2).

25. Kharat, P. S., L. B., Ghoble, K. B., Shejule, R. S. Kale and Ghoble, B.C. **2009.** Impact of TBTCl on total protein content in cresh water prawn, Macrobrachium kistnensis. *Middle- East J. Sci. Res*., **4**(3): 180- 184.

26. Doganlar, Z. B., K. Demir, H. Basak and Gul, I. **2010.** Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars. *Afr. J. Agric. Res*., **5**(15): 2056-2065.

27. Smirnoff, N. **2005.** *Antioxidants and reactive oxygen species in plants*. Blackwell Publishing Ltd.317pp.

28. Zhang, L., Tan, Q., Lee, R., Trethewy, A., Lee, YH. and Tegeder M. **2010.** Altered xylemphloem transfer of amino acids affects metabolism and leads to increased seed yield and oil content in Arabidopsis. *Plant Cell*, **22**: 3603-3620.

29. Singh, SB., Davis, AS., Taylor, GA., Deretic, V. **2006**. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science*. 2006; **313**: 1438–1441. [PubMed: 16888103]