The Study of Congo Red and Brilliant Green Dyes Effect On *Lemna minor* L. Anatomical Characteristics

Sadiq Kadhum lafta Al-Zurfi
Ecology Department, Faculty of Science
University of Kufa, Al-Najaf, Box 21, Najaf, Iraq
sadiqk.alzurfi@uokufa.edu.iq

Nidal A. Albdairi
Biology Department, Faculty of Education for Girls
University of Kufa, Iraq.

Saba A. Almadany
Biology Department, Faculty of Education for Girls
University of Kufa, Iraq.

Abstract-This study aimed to test the effect of Congo red and Brilliant green dyes on anatomical Characteristics of Lemna minor L. plant. The plant was collected from Euphrates River-Iraq and acclimation in the laboratory during 15-days, cultured in a plastic containers with triplicate frequencies and exposed to different concentrations of Congo red and Brilliant green dyes (0.01, 0.04, and 0.07 µg/l). Flowed the anatomical variations during (1, 7, 14, and 21) days. Anatomical analyses of *Lemna minor* plant revealed several changes in the leaves of plants submitted to different concentrations treatments compared with control. Exposure to dyes texture leads to a reduction in the size of blade thickness, number of conducting elements, the reduced cell size of the epidermis and aerenchyma tissue. Stems undergo shifts in size, body and arranging of cortical parenchyma cells, plants of the treatment with additional contamination had widened cell spaces in the cortex of parenchyma cells, reduced in vascular bundles.

Keywords- Anatomy, Brilliant, Congo, Dye, Lemna.

I. INTRODUCTION

Although texture dyes have taken place in the aquatic ecosystem for further than a decade, researches concerning texture dyes toxicity in these plants are limited. Water-related industrial pollutants are among the most dangerous pollutants. They are liquid products consisting of organic or inorganic substances or both, as well as toxic substances. These industrial pollutants are put into water estuaries, whether in rivers, seas or oceans, which cause the elimination of species and the deposition of living organisms. [1, 2]. Almost 40,000 dyes and pigments are listed which contains upon 7000 diverse chemical structures. Nearly all of them are fully resistant to biodegradation processes [3]. Organic dyes and acids are the most important pollutants in the wastewater of textile industries. Used in the food, pharmaceutical, paper, printing, leather and cosmetics industries, so many of these dyes find their way into the environment through sewer pipes. Compounds are of concern to the environment [4] as well as normal and synthetic polymers that cause high pH values due to the use of alkalis, surfactants and high-temperature rates resulting from wet processes such as washing and rinsing as well as the rise in total suspended solids and total dissolved minutes. Water from textile industries contains chlorine compounds that reduce the amount of oxygen dissolved in water, as well as its ability to react with other compounds and form complex chlorine salts that are harmful to organisms. The quality of water liable for the decomposition processes bio [5]. Azo dyes are the largest class of synthetic aromatic dyes composed with one or more (N=N) groups and sulfonic(-SO-3) groups with lots of commercial interest [6]. Azo compounds appear about two-thirds of all synthetic dyes. Their usage in pharmaceutical industry has many purposes [7]. The inappropriate disposal of azo dyes to water bodies currently causes great concern since it can disturb the ecosystem and constitutes a potential environmental and health problem due to their toxicity and carcinogenicity to plant and animals [8, 9]. Many azo dyes and their reductively cleaved products as well as chemically concerning aromatic amines are reported to affect human health, causing allergies and

other human maladies [10]. The effects of Malachite Green (MG) on plant species have been mostly examined using seed germination and the plant seedling stage, with germination and seedling development being generally inhibited [11, 12].In other study that Malachite Green (MG) contamination in water also caused negative effects to B. chinensis, in particular at concentrations greater than 1 mg/From the results, the negative effects of MG were strongly evidenced on root growth which was reduced by 50% upon exposure to MG of 2 mg/L and 4 mg/L compared to the control or 1 mg/L MG treatments [13]. Considering the potential risk of accidents due to industrial dyes activity and the importance of plant for the ecosystem this study aims to the presented study anatomy and doing cross-sections of plant organs of Lemna minor exposure with dyes for 21 days, which absorbed dyes are accumulated.

II. MATERIAL AND METHODS

The study was conducted from January to July 2019 in the Department of Ecology lab. The floating plant Lemna minor was collected from Euphrates River. The plant used have been chosen due to their wide distribution throughout for being native to the Euphrates river and typical of the aquatic ecosystem. Plant were washed various times with tap water then distilled water in order to take off any small invertebrates and algae [14]. Plant were adapted for 14 days in tap water. After acclimatization, they were exposed to the selected concentration at (0.01, 0.04, 0.07) µg/l for Red dye (Congo) and Green dye (Brilliant) at a time interval of 1, 7, 14 and 21 days. Triplicate batch tests were conducted in plastic container of dimensions (30*20*30) cm. Chosen dyes concentration was added in each container from prepared stock solution. About 20 gm plant was kept in each container, the water level was adjusted. All containers were exposed to light adequately (500 lux) and fixed temperature (28 C̊) for detention time of 21days. Every day, tap water was added to maintain the same level in each container. After whenever interval the plant was collected and washed with deionized water to take off any metal adhering to its surface. The washed plant samples were carefully dried. The anatomical analyses of Lemna minor leaves were carried out after 21 days of exposure to texture dyes (Red Congo and Brilliant Green), by sampling leaves presenting visible alterations and leaves in the control group and storing them in 70% ethanol. Transversal parts were performed within bulb and the median region of leaves for the mounting of semi-permanent microscope slides. The histological parts were filtered with sodium hypochlorite, washed with filtered water, stained with Safra-blau coloration, placed upon microscope slides and cover slips with a drop of glycerin, and embedded with nail polish. Watching of the histological parts were completed using an optic microscope [15].

III. RESULTS

The problem of dyes in industrial wastewater has received "great" attention because of its carcinogenic and mutagenic effect of humans and other organisms as well as the toxic effect of plants and microorganisms [8]. Plate 1 showed Leaf longitudinal sections of Lemna minor at adding industrial dye (Congo Red) 0.01µg/l concentration where that the A plate represent Lemna minor plant, B represents plant as Control during the first day, C represents Plant in 7th day, show shrivel in the surface of upper and lower epidermis and increase cell walls thickness, D represents plant during 14th day, where the surface of the epidermis increased with a minimize of leaves thickness and reduction in mesophyll tissue and initiation of aerenchyma dissolution (Table 1). E and F represent plant in 21st day, where most of the mesophyll layer was occupied by aerenchyma tissue and curvature of the inner surface of the epidermis. Clear plate 2 leaf longitudinal sections of Lemna minor under X40, X100 and X400 magnification with scale bars (100, 250 and 1000) µm respectively, after adding texture dye (Congo Red) with 0.04. µg/l Where the A represent Lemna minor plant, B represent Control in the first day, C represent plant in 7th day show the plant begins to fall into the aquarium bottom and D represent plant in 14th and 21st day, where increases the surface of the epidermis curvature with shrivel in the surface of the upper and lower epidermis and receding the thickness of the mesophyll layer (Table 1) and increase the thickness of cell walls. E and F = plant in 100X and 400X magnification, where most of the mesophyll layer was occupied with industrial dye.

Plate 3 clear leaf longitudinal sections (B,C) and cross-section(E) of Lemna minor under X40 after adding texture dye (Congo Red) with 0.07 µg/l that A represent Lemna minor plant , B represent Control in the first day, C and D represent plant during (7th and 14th) day , show the plant appear yellowish and sink in pole , where the volume of mesophyll doubled and an increase in the aerenchyma number with a small size and E represent plant in 21st day , where diameter of the vascular bundle become smaller (Table 1) with curvature of the outer and inner roof of the epidermis.

While plate 4 clear Longitudinal sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Brilliant Green) at concentration of 0.01 µg/l that A represents Lemna minor plant , B represents Control in the first day, C represents plant in 7th day, Show shrivel in epidermis and plants have yellowish appearance , D represents plant during the 14th day, where the surface of the epidermis increased with a minimize of leaves thickness and reduction in mesophyll tissue and initiation of aerenchyma dissolution (Table 1),and E represents plant in 21st day, where most of the mesophyll layer was hardened and lack of aerenchyma tissue and multiple layers of the epidermis and small size of a plant in general , So note that the plant at the end of the experiment fell to the bottom.

Plate (5,6) clear the longitudinal sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Brilliant Green) at concentration of 0.04 µg/l. plates showed that A represents Lemna minor plant , B represents in the first day, C represents plant in 7th day. The plant begins to drop down into the aquarium bottom , D, E and F represents plant in 21st day, Where increases the roof of the epidermis curvature with shrivel in the roof of the upper and lower epidermis and receding the thickness of the mesophyll layer and increase the thickness of cell walls. E and F represents plant in 100X and 400X mag knsz';vcxnification, where most of the mesophyll layer was occupied with texture dye.

Plate 7 clear Cross sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Brilliant Green) at concentration of 0.07 µg/l that A represents Lemna minor plant , B represents plant Control in the first day, C represents Plant in 7th day showed the epidermis begins to shrivel and accumulation of dye at outside of cell wall , D represents plant during 14th day where most of mesophyll converted to hypodermis cell with limited of aerenchyma cell ,and E represents plant in 21st day, where increases the shrivel in the roof of the upper and lower epidermis and receding the thickness of the mesophyll layer , Where most of the mesophyll layer was occupied with industrial dye. plant leaves at the ending of the experiment come to be completely disintegrates. It remains a thin layer of the epidermis and the plant is dead.

IV DISCUSSION

Emergent and floating plants are important to fish, wildlife, and humans. Some articles have briefed the worth of these plants for fish and wildlife [16, 17, 18]. The contamination of leaves of aquatic plants with dyes would cause the contamination of fish. The Lemna minor used as feed on the fish production in polyculture system [19] and thus, the death of those plants could cause a drastic reduction in the feeding options for this fish and due to their dense mass of roots for some aquatic plant as Eichhornia crassipes provide habitat for fish, fingerlings, insects, and other aquatic organisms [20]. The lowering of thickness and even the loss of palisade parenchyma tissue resulting from exposure to the dye indicate that even if the plant produces new leaves they will have their functions compromised. Were in another study [21] showed the high concentration of heavy metal causes general the lack of thickness of leaf and tortuosity of the epidermis surface, airspace decreased in number and increased in the area, the disintegration of mesophyll cell and epidermis cell increased in thickness. Palisade parenchyma is found generally in the adaxial or upper roof of the leaves and consists of areas of elongated cells, rich in chloroplasts, placed perpendicularly to the surface of the foliar limb, presenting intercellular spaces [22]. This is the major tissue responsible for the photosynthesis of tracheophytes and presents high plasticity in response to environmental factors for a wide diversity of plants [23], including L.minor, as demonstrated in this study. Our conclusion found in [17], which have been reported that size variations in epidermal tissues in response to water pollution conditions. Increased thickness of the abaxial and adaxial, as caused by heavy metals, might be related to adsorption of metals in the cell walls, constituting an alternative pathway for allocation of these ions and preventing their translocation to photo-synthetic tissues [24]. The inhibition of root growth causes a reduction in nutrient absorption, increasing the plant's stress and hampering their development. Although in the present study the roots of L.minor were not measured, Plants also secrete some chemical compounds known as exudates to initiate and support the root bacterial community to perform degradation (photostimulation) [25].

These results indicate that texture dye (Brilliant Green) cause more severe effects on Lemna minor than Red Congo. On the other hand, the insoluble part of the dyes reaching the root system where the pollutant becomes available for absorption by the plants. The implication of such differences in the case of texture dyes in aquatic ecosystems is that Brilliant Green could cause immediate damage to Lemna minor, while Red Congo to L. minor damage would be evident only after prolonged exposure.

Thus, even though texture dyes may not be immediately lethal to fish, it would cause the loss of habitat and the accumulation of dead organic matter in the environment. The decomposition of this material will further decrease oxygen availability in aquatic ecosystems. Heavy metals would also contaminate the dead organic matter [26] and when decomposition takes place toxic elements may be incorporated into the trophic chains via the ingestion of plant material by invertebrates, preferential food items of several fish species.

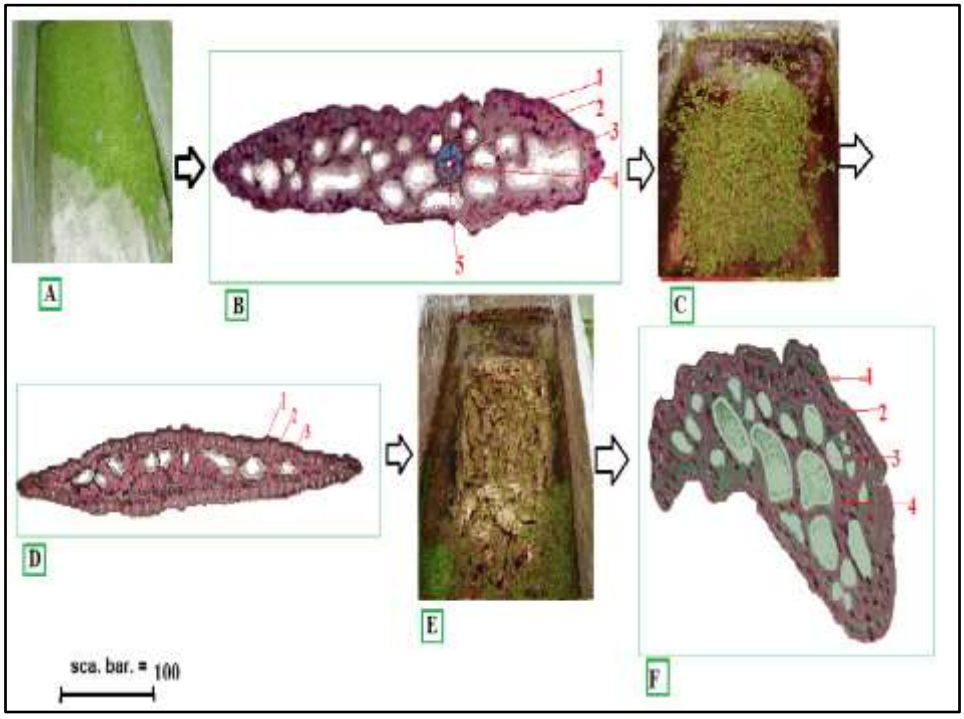

Plate (1): Longitudinal sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Congo Red) at concentration of 0.01 µg/l . The images are shown in X 40 magnification with scale bars =100 µm.1=Epidermis cells, 2=Parenchyma tissue, 3=Aerenchyma tissue, 4=vascular bundle, 5= Central pore of Vascular bundle

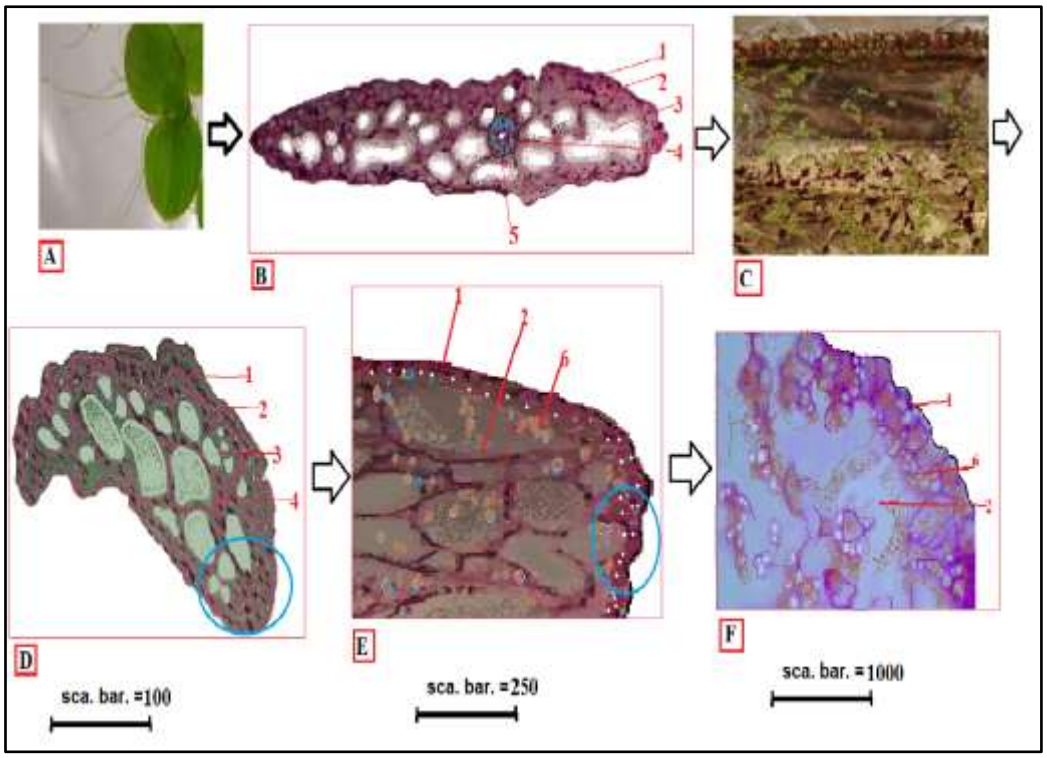

Plate (2): Longitudinal sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Congo Red) at concentration of 0.04 µg/l. The images are shown in X 40, X100 and X400 magnification with scale bars = 100, 250 and 1000 µm:1=Epidermis cells, 2=Parenchyma tissue, 3=Aerenchyma tissue, 4=Vascular bundle, 5= Central pore of Vascular bundle, 6= Accumulation of dye

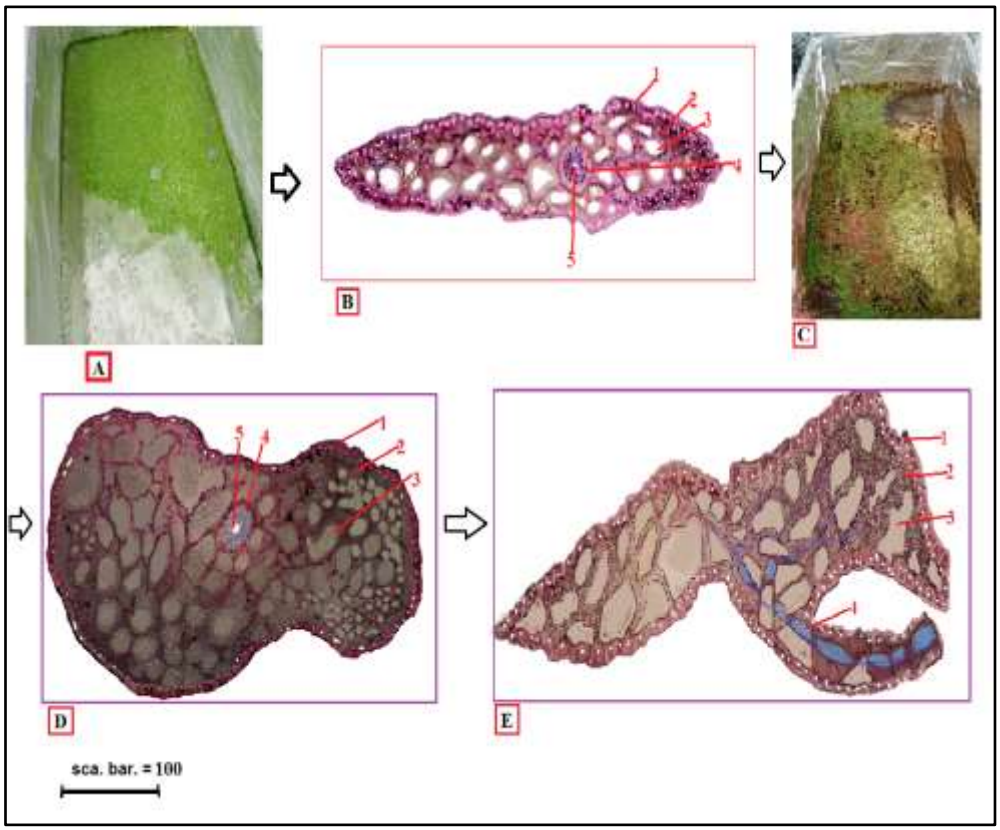

Plate (3): Longitudinal sections (B) and cross section in (D,E) of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Congo Red) at concentration of0.07 µg/l. The images are shown in X40 magnification with scale bars =100 µm. 1=Epidermis cells, 2= Parenchyma tissue, 4= Aerenchyma tissue, 5=Vascular bundles, 6= The central pore of vascular bundle.

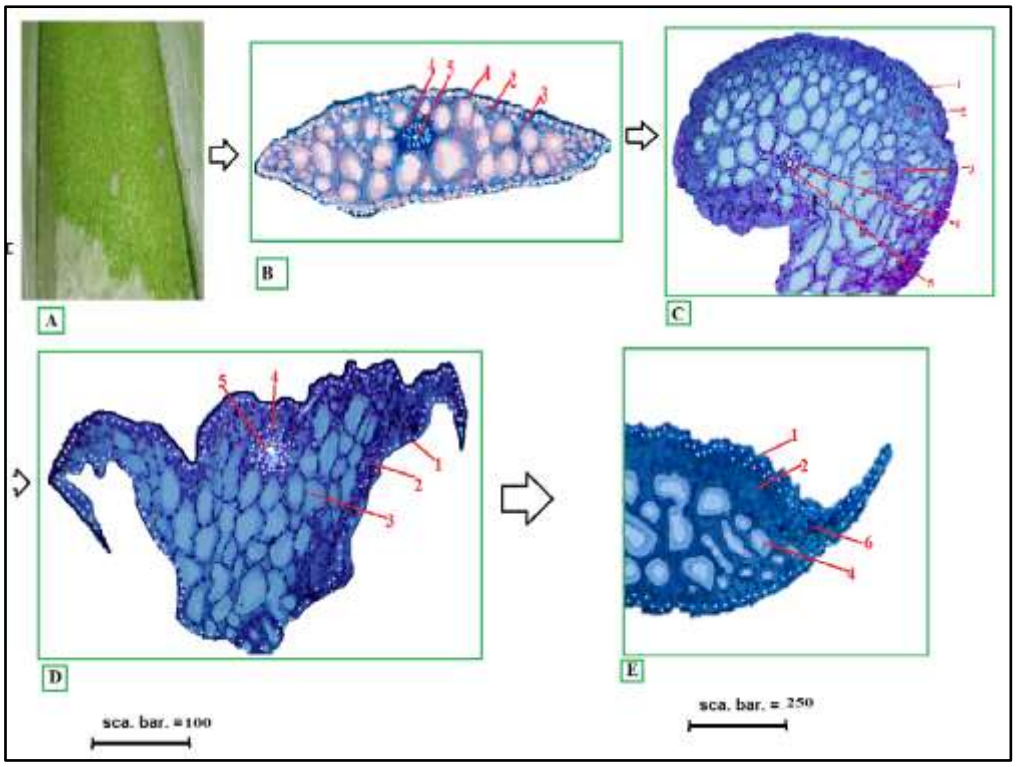

Plate (4): Longitudinal sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Brilliant Green) at concentration of 0.01 µg/l.:1=Epidermis cells, 2=Parenchyma tissue, 3=Aerenchyma tissue, 4=vascular bundle, 5= Central pore of Vascular bundle, 6=multiple epidermis

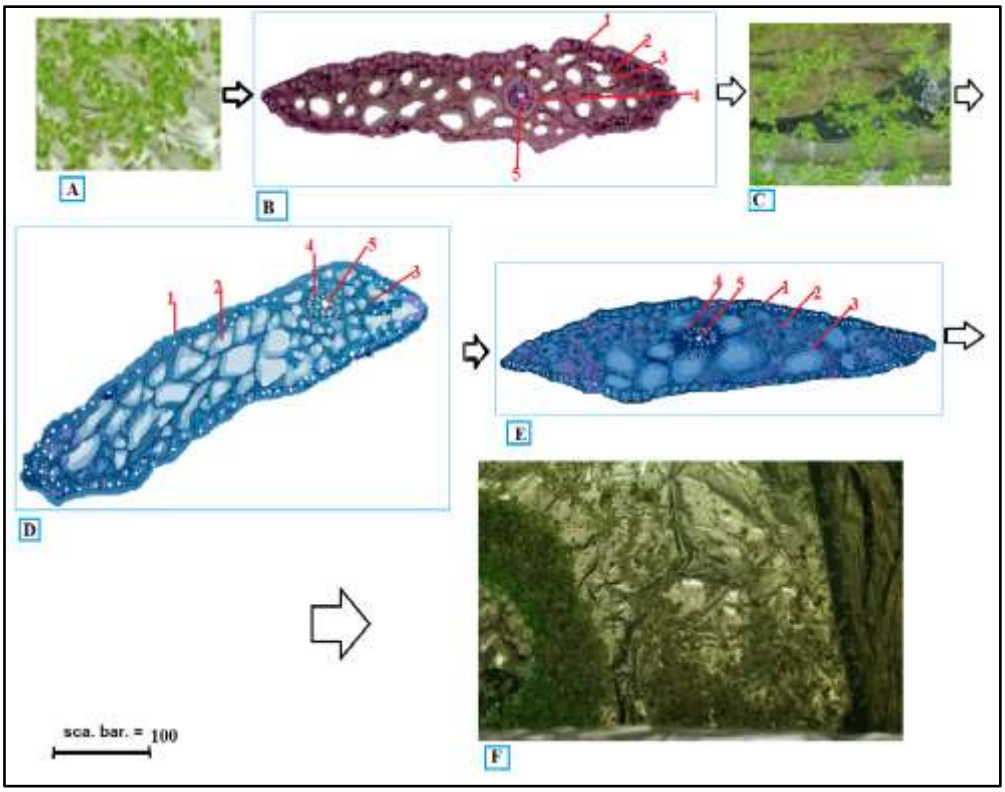

Plate (5): Longitudinal sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Brilliant Green) at concentration of 0.04 µg/l . The images are shown in X 40 magnification with scale bars =100 µm.:1=Epidermis cells, 2=Parenchyma tissue, 3=Aerenchyma tissue, 4=vascular bundle, 5= Central pore of Vascular bundle

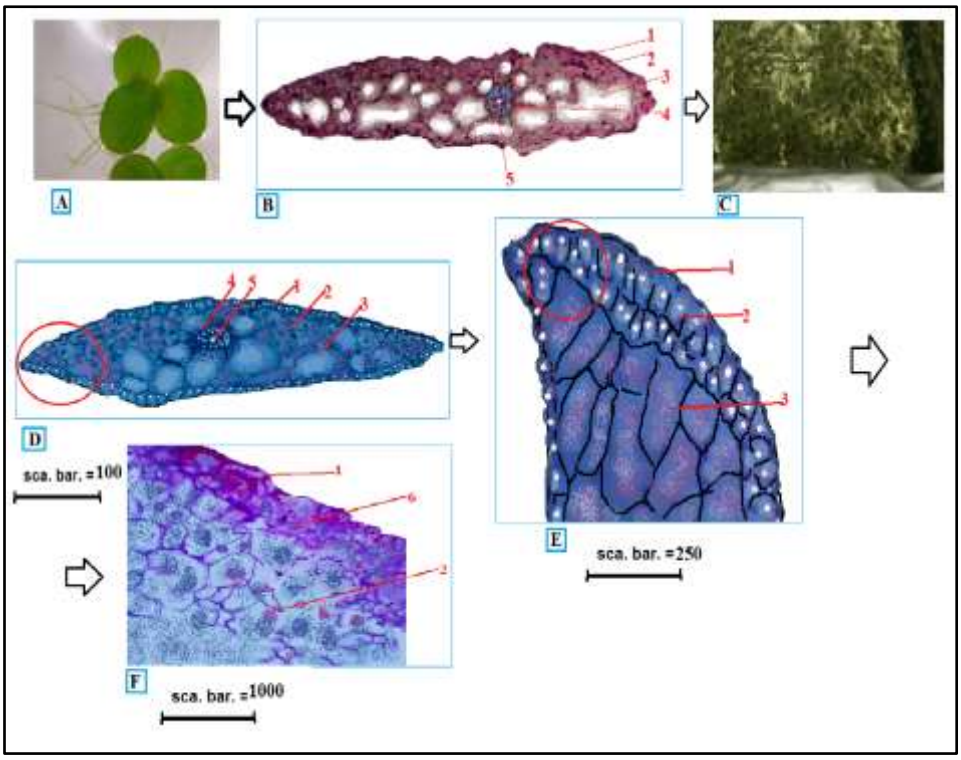

Plate (6): Longitudinal sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Brilliant Green) at concentration of 0.04 µg/l . The images are shown in X40, X100 and X400 magnification with scale bars = 100, 250 and 1000 µm.:1=Epidermis cells, 2=Parenchyma tissue, 3=Aerenchyma tissue, 4=Vascular bundle, 5= Central pore of Vascular bundle , 6= Accumulation of dye

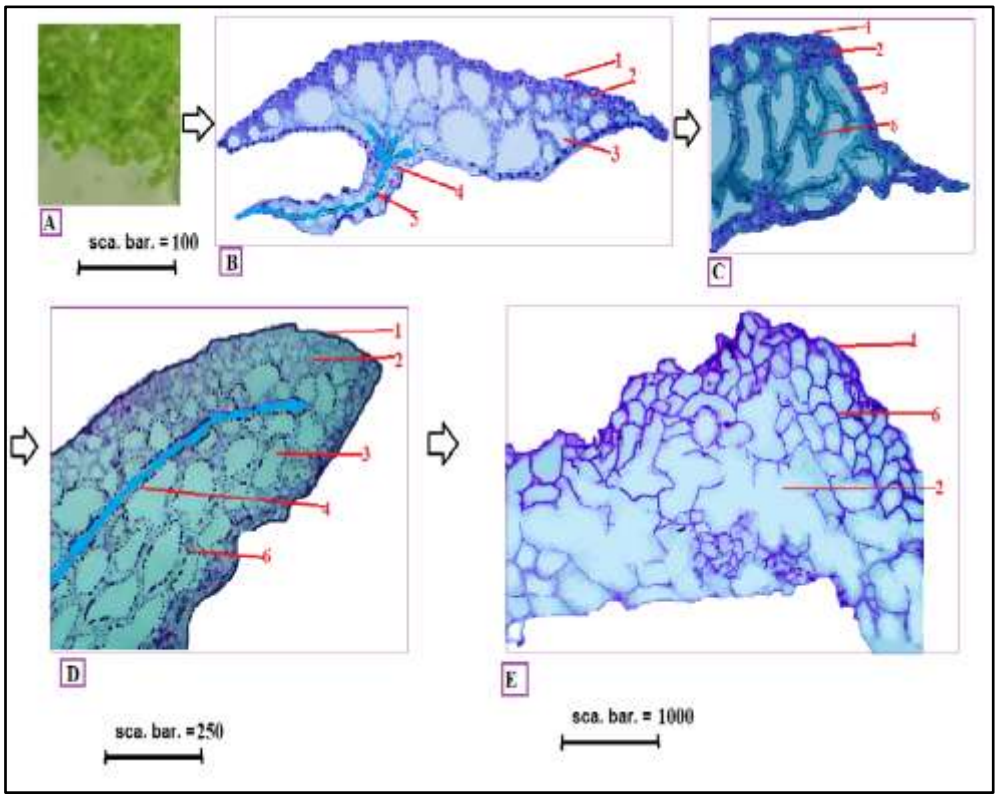

Plate (7): Cross sections of Lemna minor leaf after (1,7,14 and 21) day of exposure to texture dye (Brilliant Green) at concentration of 0.07 µg/l. The images are shown in X40, X100 and X400 magnification with scale bars = 100, 250 and 1000 µm respectively:1=Epidermis cells, 2=Parenchyma tissue, 3=Aerenchyma tissue, 4=Vascular bundle, 5= Central pore of Vascular bundle , 6= Accumulation of dye.

Table 1- Measurement of leaf in Lemna minor in millimeter

Dye type	Day	Concentration (µg/L)	Vascular bundle diameter	Total leaf thickness	Lower Epidermis Thickness	Aernchema thickness	Upper Epidermis thikness	Mesophyle thickness
Red Congo	7	0.01	0.83	0.75	0.21	0.14	0.22	0.36
	14		0.85	0.74	0.25	0.15	0.26	0.34
	21		0.8	0.72	0.23	0.16	0.233	0.35
	7	0.04	0.64	0.85	0.53	0.22	0.52	0.24
	14		0.62	1.1	0.54	0.22	0.53	0.25
	21		0.63	1.12	0.55	0.22	0.54	0.26
	7	0.07	0.41	0.8	0.32	0.83	0.31	0.54
	14		0.47	0.78	0.33	0.8	0.27	0.55
	21		0.4	0.71	0.31	1	0.33	0.57
P.value(0.05) Con.			0	0	0	0	0	0
Brilliant green	7	0.01	0.32	0.66	0.42	0.22	0.41	0.52
	14		0.31	0.64	0.41	0.24	0.43	0.53
	21		0.31	0.64	0.42	0.22	0.41	0.53
	7	0.04	0.56	0.51	0.31	0.27	0.527	0.37
	14		0.52	0.53	0.31	0.29	0.503	0.38
	21		0.51	0.54	0.32	0.26	0.5	0.33
	7	0.07	0.53	0.46	0.23	0.25	0.32	0.15
	14		0.51	0.41	0.25	0.22	0.327	0.18
	21		0.59	0.41	0.27	0.21	0.307	0.12
P.value(0.05) Con.			0	0	0	0.031	0	0
Without Dyes	7	Control	1.5	1.72	0.17	0.1	0.15	0.32
	14		1.4	1.75	0.14	0.3	0.13	0.35
	21		1.5	1.72	0.12	0.2	0.117	0.35

IV.CONCLUSION

This study concluded that Congo red and Brilliant green dyes could affect the anatomical characteristics of *Lemna minor* L. plant. Anatomical analyses of *Lemna minor* revealed several changes in the leaves of plants submitted to different concentrations of treatments compared with control. Exposure to dyes texture leads to a reduction in the size of blade thickness, number of conducting elements, the reduced cell size of the epidermis, and aerenchyma tissue. Stems undergo shifts in size, body, and arranging of cortical parenchyma cells, plants of the treatment with additional contamination had widened cell spaces in the cortex of parenchyma cells, reduced in vascular bundles.

V. AKNOWLEGEMENT

We would like to thankful Department of Ecology-Science Faculty and Biology Department, Faculty of Education for Girls- University of Kufa, Iraq to funding this work. Also, we would be thankful Prof Dr. Mohammed Jawad Al-Haidarey for supporting the team work in the lab of Advance Ecology.

REFERENCES

[1] Danazumi, S., & Bichi, M. H. (2010). Industrial Pollution And Heavy Metals Profile Of Challawa River In Kano, Nigeria. Journal Of Applied Sciences In Environmental Sanitation, 5(1).
[2] Agarwal, S. K. (2009). Pesticide Pollution (Vol. 3). APH Publishing Corporation New Delhi.
[3] Rehman, R., Murtaza, S., Zaman, W., & Shafique, U. (2012). Comparative Removal Of Congo Red Dye From Water By Adsorption On Grewia Asiatica Leaves, Raphanus Sativus Peels And... J. Chem. Soc. Pak, 34(1), 112.
[4] Saratale, R.G.; Saratale, G.D.; Chang, J.S. & Govindwar, S.P. (2011). Bacterial Decolorization And Degradation Of Azo Dyes: A Review. Journal Of The Taiwan Institute Of Chemical Engineers, Vol. 42, No. 1, Pp. 138–157, ISSN 1876-1070.
[5] Gosavi, V. D., & Sharma, S. (2014). A General Review On Various Treatment Methods For Textile Wastewater. J Environ Sci Comput Sci Eng Technol, 3, 29-39
[6] Barragán, B. E., Costa, C., & Márquez, M. C. (2007). Biodegradation Of Azo Dyes By Bacteria Inoculated On Solid Media. Dyes And Pigments, 75(1), 73-81.
[7] Gičević, A., Hindija, L., & Karačić, A. (2019). Toxicity Of Azo Dyes In Pharmaceutical Industry. In International Conference On Medical And Biological Engineering (Pp. 581-587). Springer, Cham.
[8] Puvaneswari, N., Muthukrishnan, J., & Gunasekaran, P. (2006). Toxicity Assessment And Microbial Degradation Of Azo Dyes.Indian Journal Of Experimental Biology,44:618-626.
[9] Banat IM, Nigam P, Singh D, Marchant R.(1996) Microbial Decolorization Of Textile-Dye-Containing Effluents: A Review. Bioresour Technol;58:217-227.
[10] Chung, K. T. (2016). Azo Dyes And Human Health: A Review. Journal Of Environmental Science And Health, Part C, 34(4),

233-261.
[11] Kagalkar, A. N., Jadhav, M. U., Bapat, V. A., & Govindwar, S. P. (2011). Phytodegradation Of The Triphenylmethane Dye Malachite Green Mediated By Cell Suspension Cultures Of Blumea Malcolmii Hook. Bioresource Technology, 102(22), 10312-10318.
[12] Gopinathan, R., Kanhere, J., & Banerjee, J. (2015). Effect Of Malachite Green Toxicity On Non Target Soil Organisms. Chemosphere, 120, 637-644
[13] Matpang, P., Sriuttha, M., & Piwpuan, N. (2017). Effects Of Malachite Green On Growth And Tissue Accumulation In Pak Choy (Brassica Chinensis Tsen & Lee). Agriculture And Natural Resources, 51(2), 96-102
[14] Lytle, C. M. And B. N. Smith (1995). Seasonal Nutrient Cycling In*Potamogeton Pectinatus* Of The Lower Provo River. *Great Basin Naturalist*, **55** : 164-168.
[15] Thammathaworn, A. (1996). *Handbook By Paraffin Method*. Department Of Biology, Faculty Of Science, Khon Kaen Universality, Thailand.
[16] Killgore, K. J., Dibble, E. D., & Hoover, J. J. (1993). Relationships Between Fish And Aquatic Plants: A Plan Of Study (No. WES/MP/A-93-1). Army Engineer Waterways Experiment Station Vicksburg Ms. Environmental Lab
[17] Melo, H. C. D., Castro, E. M. D., Soares, Â. M., Melo, L. A. D., & Alves, J. D. (2007). Alterações Anatômicas E Fisiológicas Em Setaria Anceps Stapf Ex Massey E Paspalum Paniculatum L. Sob Condições De Déficit Hídrico. Hoehnea, 34(2):145-153.
[18] Pelicice, Fm., Thomaz, Sm. And Agostinho, AA. (2008). Simple Relationships To Predict Attributes Of Fish Assemblages In Patches Of Submerged Macrophytes. Neotropical Ichthyology, Vol. 6, P. 543-550.
[19] Talukdar, M. Z. H., Shahjahan, M., & Rahman, M. S. (2012). Suitability Of Duckweed (Lemna Minor) As Feed For Fish In Polyculture System. International Journal Of Agricultural Research, Innovation And Technology, 2(1), 42-46.
[20] Junk, W. J. & C. Howard-Williams, (1984). Ecology Of Aquatic Macrophytes In Amazonia. In Sioli, H. (Ed.), The Amazon Limnology And Landscape Ecology Of A Mighty Tropical River And Its Basin. W. Junk, Dordrecht: 269–293.
[21] Al-Zurfi, S. K. L., Alisaw, A. Y., & Al-Shafai, G. A. A. (2018). Bioaccumulation Of Some Heavy Metals By The Aquatic Plant Lemna Gibba In Vitro. Research Journal Of Pharmacy And Technology, 11(10), 4229-4236
[22] Souza, L. A., (2003). Morfologia E Anatomia Vegetal: Célula, Tecidos, Órgãos E Plântula. Editora Da Universidade Estadual De Ponta Grossa, Ponta Grossa.
[23] Justo, C. F., Â. M. Soares, M. L. Gavilanes & E. M. De Castro, 2005. Leaf Anatomical Plasticity Of Xylopia Brasiliensis Sprengel (Annonaceae). Acta Botanica Brasilica 19(1): 112–123.
[24] Gomes, M. P., Marques, T. C. L. L. D., E Melo, S., Nogueira, M. D. O. G., Castro, E. M. D., & Soares, Â. M. (2011). Ecophysiological And Anatomical Changes Due To Uptake And Accumulation Of Heavy Metal In Brachiaria Decumbens. Scientia Agricola, 68(5), 566-573.
[25] Imron, M. F., Kurniawan, S. B., Soegianto, A., & Wahyudianto, F. E. (2019). Phytoremediation Of Methylene Blue Using Duckweed (Lemna Minor). Heliyon, 5(8), E02206
[26] Osuji, L. C. & S. O. Adesiyan, (2005). Extractable Hydrocarbons, Nickel and Vanadium Contents Of Ogbodo-Isiokpo Oil Spill Polluted Soils In Niger Delta, Nigeria. Environmental Monitoring and Assessment 110: 129–139.