

## Effects oil pollution on the some Biochemical and anatomical characteristics of *Ceratophyllum demersum* and *Myriophyllum spicatum*

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### Abstract

This study examines the effects of several crude oil concentrations on specific biochemical and anatomical characteristics of two submergent aquatic macrophytes: *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. The experimental groups included control (0% crude oil) and plants treated with crude oil at rates of 0.2%, 0.3%, 0.4% and 0.5%. Results indicate a significant decline in total chlorophyll concentration in *C. demersum* at the 0.5% crude oil treatment (1.0424 mg/L) and in *M. spicatum* at the same concentration (0.3552 mg/L). Anatomical examination of stems revealed negative impacts of all crude oil concentrations tested, especially the higher rates of 0.4% and 0.5%. However, the 0.2% and 0.3% concentrations were less detrimental than the higher doses, suggesting some capacity of these plants to withstand increased crude oil levels. This was reflected in a reduction in aerenchyma cell size, with some them disappearing completely. In comparison to *M. spicatum*, *C. demersum* displayed greater tolerance to high crude oil concentrations. Additionally, both species experienced reduced stem diameter, diminished cortical thickness and decreased size of the vascular tissues.

Keywords: Crude oil contamination, Aquatic macrophytes, Biochemical and anatomical responses

### Introduction

Crude oil pollution is an important environmental problem affecting the physical, chemical and biological qualities of natural marine and land ecosystems. UNEP (2019) defined Pollution as a change in air, water and soil, so physical or chemical or biological factors would lead to deterioration of the qualities of the environment and life forms that'd cause severe harm. Also, a Pollutant is a chemical substance that becomes a pollutant through either human intervention or natural processes in certain quantities that'll contaminate the air, water

and soil to levels that pose environmental risks or pose serious harm to the well-being of plants, humans and other animals. (Bhatia, 2009) Oil spill pollution is considered a major risk for environmental disasters, affecting multiple environmental elements such as aquatic species, fish, turtles, birds, sea mammals and vegetation found in the area, with widespread socioeconomic impact in addition to the environmental (Hinojosa *et al.*, 2004). The problem arose due to the growth and progression in the technology applied. Today, the main source of energy

worldwide is crude oil. Oil spills occur globally across diverse environments in the world, in various amounts and levels and thus poses multiple risks to the environment. Spillage of oil may occur throughout its lifecycle: extraction, transportation and consumption. (Al-Moaikal *et al.*, 2012). Besides, the exploration of oil, the transportation of crude oil or oil products, refining processes, the extraction and consumption of petroleum contribute significantly to widespread pollution of terrestrial and aquatic environments, with considerable impact on the soil resulting from oil accumulation and presence of petroleum hydrocarbon compounds (Ma *et al.*, 2018). Crude oil consists of multiple compounds of hydrocarbon derivatives which show variations in appearance and composition according to the field source, but it consists of many derivatives such as hydrocarbon molecules that's utilized as a lubricant, as a fuel for ships and airplanes, as a fuel for heating in residences, as cosmetics, inks, medications, fertilizers, Plastics, chemicals used in the cosmetic industry and many other usages (Aljamali & Salih, 2021). Pollution is brought on by various types of human activity which lead to release of petrol hydrocarbons, such as cooling agents, lubrication oil as coolant lubricants, grinding metals, the car wash activity and related industries that supply chemicals required for washing vehicles and activities done by chemical plants where compounds are obtained or produced via distillation of oil (Bayat *et al.*, 2016).

Plants form an integral component of these natural resources. Different types of vegetation may be observed and prevalent across different aquatic regions like lakes, rivers, marshes and even ponds. These may include prominent or floating or submersed aquatic species that vary in their variety, density and role as indicators of the health of an aquatic region in terms of

maintaining pure fresh water bodies or otherwise (Izzati, 2015). Some types of aquatic species have similar morphological traits, be it internal or outer tissues. Other elements such as the levels of pollutants or environmental conditions of aquatic zones (physical and chemical properties) might've varied impact on these species, whether positively or negatively. Aquatic vegetation may be defined as plants which grow in water environments, species which live part or their total life in the water. This definition includes floating plants and plants growing underwater. It also refers to seeds growing on water or anyplace adjacent to a body of water and germinating in the water (Tamire and Mengistou, 2013). *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. species are found throughout many Iraqi water regions along with the Shatt al-Arab River, especially at points where oil spill impacts are prominent. Crude oil spills lead to an increase in oxygen consumption due to cell injury to cells leading to loss of semi permeability of the cells and membranes, there's a rupture in cell contents, especially cytoplasm, leading to the influx of oil. Gas exchange in aquatic plants is hindered by hydrocarbon compounds from crude oil primarily by the creation of a surface film, which acts as a physical barrier to the diffusion of oxygen from the atmosphere to the water, as well as alters the physical/chemical nature of the surrounding water. gas exchange in submerged plants such as *Ceratophyllum demersum* and *Myriophyllum spicatum* (L.) are primarily through the plant surface itself, then internally through the aerenchyma, therefore stomata are irrelevant to their means of respiration and gas exchange. Hydrocarbon compounds present in crude oil impair gas exchange in aquatic plants primarily by forming surface films that reduce oxygen diffusion and alter the physicochemical properties of the surrounding water. In submerged

macrophytes such as *C. demersum* and *M. spicatum*, gas exchange occurs predominantly through direct diffusion across plant surfaces and internal transport via aerenchyma tissues rather than stomatal regulation. Crude oil contamination disrupts membrane permeability and limits the availability of dissolved oxygen and carbon dioxide, thereby impairing respiratory and photosynthetic processes. Additionally, hydrocarbon exposure can induce oxidative stress, leading to damage of chloroplast structures and inhibition of photosynthetic efficiency. Consequently, both photosynthesis and overall metabolic activity are significantly reduced (Baker, 1970).

Akapo *et al.* (2011) studied phenotypic and anatomical effects of crude oil pollution on *Pistia stratiotes*. It found crude oil toxic to plants in all concentration effects. Crude oil was able to bring about severe cellular damage as well as some cellular structure and anatomy modifications especially at the apex. Baruah *et al.* (2014) recorded the effect of crude oil on chlorophyll contents and anatomical morphology in the plant *Cyperus brevifolius* (Rottb) which showed a decrease in the chlorophyll contents with increasing crude oil concentration and revealed major distortion of the structural anatomy in high oil concentrations. Lorestani *et al.* (2014) recorded that light crude oil contamination drastically reduced the percentage of germination, the rate of germination and the stem length. Anatomy of plants reveals damages in vascular bundles and cracks in cells of the skin. Punwong *et al.* (2017) in their study concluded that crude oil contamination effects anatomical structures of *Terminalia catappa* L. A substantial increase in epidermis's height, epidermis's thickness, epidermal cells' width and the height of a specific septum in the median area and increases in a number of epidermis layers were recorded. Olanonont *et al.* (2018) study on physiological responses and anatomy to

oil on a plant on a beach. It determined that a significant decrease occurred in vessel bundle height, leaf carotenoid thickness, leaf bundle width and height, axial stomata density and cell width (mean), when oil spread over its leaves. Oil absorption changed the anatomical features of plant leaves in this species. Olanonont *et al.* (2020) studied the effect of crude oil contamination on plant growth, structure and leaf anatomical features. It found that contamination with crude oil increases leaf fluidity cell walls and reduces the size of a plant cell, tissue, air pockets among them caused by the accumulation of small oil droplets found among vascular bundles. Mostafa *et al.* (2021) reported in the article that crude oil has negative impacts on the structural and functional characteristics of *Azolla pinnata*. The study showed varied carotene content throughout time, but eventually the oil contamination damaged DNA, it also impacted both the cells within the leaf tissue structure, the overall chloroplast structure and caused deformations to the outer cell of epidermal tissue of the palm leaf tissue.

Therefore, this study aims to evaluate the effects of different crude oil concentrations on the biochemical and anatomical characteristics of *C. demersum* and *M. spicatum*, with the objective of identifying species-specific tolerance mechanisms and assessing their potential as bioindicators of oil pollution in aquatic environments.

## Materials and Methods

### Experimental Design

A randomized completely design (CRD) was designed to evaluate the impact of crude oil on *Ceratophyllum demersum* and *Myriophyllum spicatum* (20% v/v). Four crude oil concentrations (0.2%, 0.3%, 0.4% and 0.5% v/v) along with a control (0%) were

selected for the experiment and each treatment had 3 replicates.

#### Preparation of Experimental Units

Glass aquaria of 20 L were washed with distilled water and air-dried before use. Then 10 L tap water was filled into each container and the water was left for 24 h for stability of physiochemical properties. The experimental oil treatments consisted of adding 20 mL, 30 mL, 40 mL and 50 mL of crude oil to separate aquaria, representing concentrations of 0.2%, 0.3%, 0.4% and 0.5% v/v. Crude oil was pre-weathered for one week under ambient conditions for removal of some of the lighter fractions of hydrocarbons by evaporation.

The mixtures of water and oil were agitated in an electric mixer at low speed to disperse the oil droplets evenly in water.

#### Plant Material and Exposure Conditions

Healthy plant shoots of *Ceratophyllum demersum* and *Myriophyllum spicatum* collected from freshwater habitat were gently rinsed with distilled water and then cut into segments of 10 cm in length. Each aquarium was stocked with 10 g dry weight of fresh plant material. Plants were left to equilibrate in tap water for 24 h before exposure. The experiment was conducted under constant environmental conditions of temperature ( $25 \pm 2^\circ\text{C}$ ) with natural day, night light cycle.

#### Anatomical Analysis

Stem sections (1 cm) were sampled at the start and end of experiment. The segments were fixed, cut in section and examined under a light microscope. Changes were noted in aeration aerenchyma development, vascular tissues of pith and cortex as well as cortical cells thickness.

#### Chlorophyll Content Determination

Total chlorophyll in the leaves was determined using the method described by

Daniel I. Arnon (1949) with slight modification. Fresh leaf (0.2 g) was homogenized in 20 mL of 80% acetone with the help of a mortar and pestle in the dim light condition for avoiding any pigment degradation. The homogenate was then centrifuged at 3000 rpm for 5 minutes and supernatant was collected. Absorbance was measured at 663 nm and 645 nm in a spectrophotometer and then calculated with the equations of standard method.

$$\text{Total Chlorophyll} = (12.7 * \text{OD } 663) + (16.8 * \text{OD } 645)$$

#### Protein:

As described by Page *et al.* (1982): Sample weights are 0.2 g of dry weight. Sample added to 5 ml of sulfuric acid. Spin to center for 20 min. Shell off the rotor on high rotation (120 m) and reheated to 90 degrees for 30 minutes. Add 4% stock acid (4 ml of pyrochloric acid in 96 ml sulfuric acid). Heated again until colorless and clear for 10 minutes. Cool down and transfer to a 50 ml plastic container for Keldal measurement to be done in the central laboratory at the College of Agriculture/University of Basra. Nitrogen to plant is calculated, multiplied by 6.25 and reported as percentage.

#### Anatomical Study

In anatomical study, plant parts (leaves and stems) were chosen and dissected into 1-cm sections, since the chosen samples were delicate, they were then fixed using FAA fixative ([5 mL] of 70% acetic acid, [5 mL] of formalin, [90 mL] of 70% ethanol, plus distilled water) for 24 hr, after which they were twice rinsed with 70% ethanol and stored in the same concentration. After 1 hour of exposure to a graded series of ethanol (80%, 90% and 95%), the plant materials were left in absolute ethanol overnight. Next, they were transferred for a half hour to each of the following solutions: mixtures of ethanol/xylene in a ratio of (1:3), (1:1), (3:1),

pure xylene for a half hour, then xylene again for 2, 3 hr. Lastly, they were put into a (1:1) mixture of melted paraffin wax/xylene and incubated in an oven set to 60°C for 4 hours. In the oven, the mixture was swapped for fresh melted paraffin wax set at 60°C and left for 24; 36 hours. Melted paraffin wax was poured into a 8-cm<sup>3</sup> plastic cube mold. The plant specimens were positioned vertically inside, allowed to harden, then placed in a basin of cold water for 20; 24 hours. The hardened blocks (specimens) were then sectioned to 15 µm thickness using a rotary microtome, the leaf and stem samples respectively, into sequential sections. These section ribbons were allowed to float on a water bath at 40°C and carefully transferred onto clean glass slides with a thin albumen layer after the excess water was drained. The glass slides were laid on a hot plate set at 30°C for wax tapes to immobilize and Xylene (24; 48 hours) followed by descending steps in ethanol (100%, 95%, 90%, 80% and 70% for 5 minutes in each). They were subsequently rinsed in 1% safranin dye dissolved in water for an hour, followed by ascending steps in ethanol (70%, 80%, 90%, 95%, 100% for 5 minutes in each). Lastly, they were briefly rinsed in fast green dye (1 g of dye dissolved in 100 mL of absolute ethanol for 2; 3 seconds) and washed with absolute alcohol, followed by pure xylene (5 minutes each) and once more with pure xylene to purge any floating debris. They were then fixated by carefully dropping Canada balsam onto the specimen just before the tape dried. The covered slide was set at room temperature and left there to dry before microscopic observation (Aledany & ALmayah, 2001).

### Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, and figures were prepared in Microsoft Excel.

All data were analyzed using a completely randomized design (CRD) with five treatments (control, 0.2%, 0.3%, 0.4%, and 0.5%) and three replicates. Measured variables included chlorophyll, protein, and anatomical parameters of *Ceratophyllum demersum* and *Myriophyllum spicatum*.

Differences among treatments were assessed by one-way ANOVA, followed by Tukey's HSD test at  $p \leq 0.05$ .

### Results :

Chlorophyll is the green pigment responsible for photosynthesis (energy production) and is found inside the plant cell in the plastids. 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.

The current results showed a decrease in the ratio of total chlorophyll of plants under study, the total chlorophyll was recorded in *C.demersum* with a maximum value 6.790 mg/L in (control) and the minimum value was 1.0424 mg/L in (0.5%) during 30 days also *M.spicatum* with a maximum value 3.0232 mg/L in (control) and the minimum value was 0.3552mg/L in (0.5%) .

The existence of crude oil in the water may be a reason to inhibit the synthesis of chlorophyll in *these* 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. explained with a high concentration of crude oil decreases the total chlorophyll content

significantly decrease. The low chlorophyll content in the plant was an indicator of environmental pollution. 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. This is also due to the fact that the crude oil in the water causes a reduced water transparency and light penetration 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.

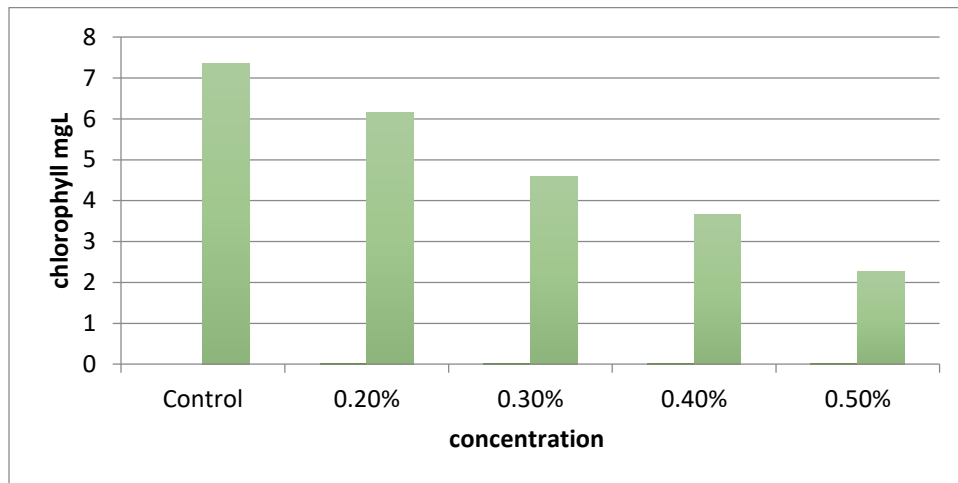


Figure 1. Variation in chlorophyll content of *Ceratophyllum demersum* under experimental conditions.

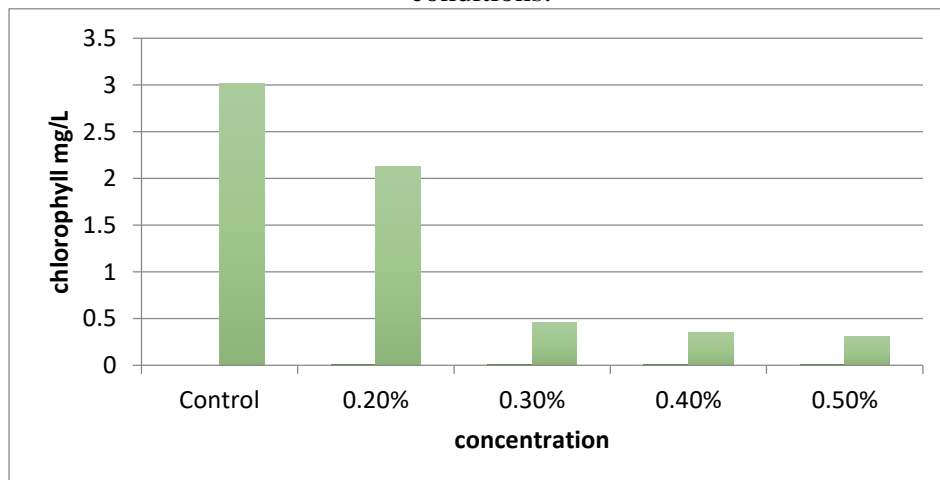


Figure 2. Variation in chlorophyll content of *Myriophyllum spicatum* under experimental conditions.

### Protein:

The results 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 ( $OH^-$ ). The current results showed a decrease in the ratio of protein of plants under study, the protein was recorded in *C. demersum* with a maximum value 20 in (control) and the minimum value was 14 in (0.5%) during 30 days also *M. spicatum* with a maximum value 14.3125 in (control) and the minimum value was 11.256 in (0.5%).

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. Studies have shown that plants can slow down the rate of protein synthesis when under stress conditions, thereby reducing protein content. 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.

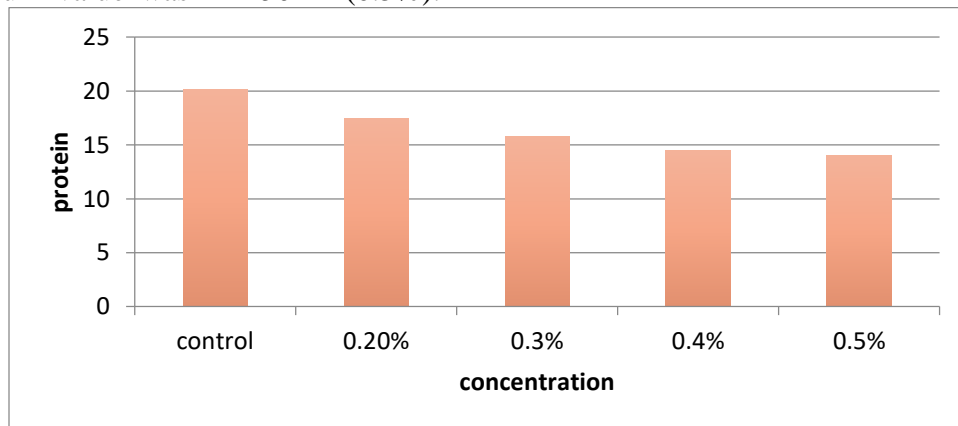


Figure 3. Variation in protein content of *Ceratophyllum demersum* under experimental conditions.

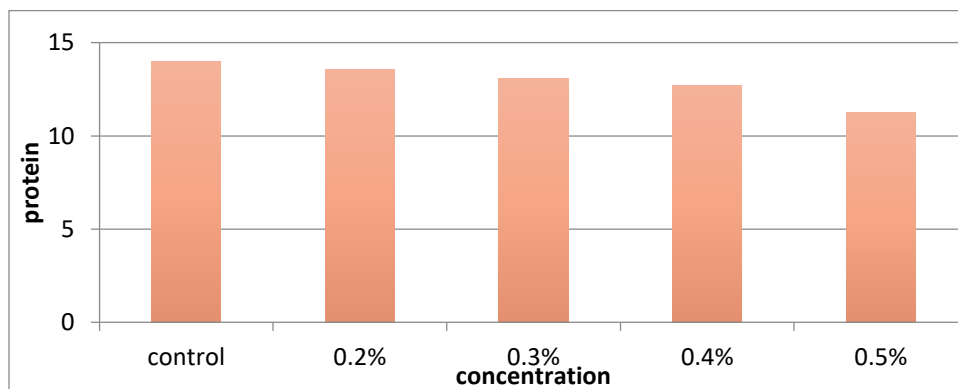


Figure 4. Variation in protein content of *Myriophyllum spicatum* under experimental conditions.

## Antamical characteristics

### 1. *Ceratophyllum demersum*

#### Control Plants

Figure 5 revealed that the higher the concentration of crude oil, the less the thickness of the cortex, the less the volume of the air spaces, and the lower the thickness of the vascular zone. The shape of the transverse section of the stems of the *Ceratophyllum* plant in the control treatment was circular as it was surrounded by a thin layer of cuticle, followed by a layer of meristem cells that consist of one row of a small square to rectangular cells, some of which are circular, followed by the cortex area, which is wide and consists of three areas: The outer cortex consists of (7-4) layers, the middle cortex, which contains the air gaps, and the inner cortex, the endodermis, that surrounds the vascular bundles. The average thickness of the outer and middle air cavities was 783.3  $\mu\text{m}$ , while the air chambers were 187.5  $\mu\text{m}$  in length and 213.75  $\mu\text{m}$  in width. And the center of the stem consisted of the vascular region, whose thickness was 653.75  $\mu\text{m}$ .

#### Plants Treated with Different Concentrations of crude oil

The Plants treated with a concentration of 0.2% of crude oil were characterized by a change in the shape of the stem from a circular to an irregular zigzag circular shape. Also, it was discovered that the thickness of the cortex was reduced at an average of 725 micrometers, along with a decrease in the average dimensions of the air chambers, as the average was 55 micrometers in length and 80 micrometers in width. As for the vascular area, its average thickness was 435, which means it fell compared to the control treatment.

From Figure 3 it was clear that plants treated with a concentration of 0.3% of crude oil

gradually began to shrink in diameter. Its shape changed from circular to oval elongated, with an average diameter of 1525 micrometers. The size of the air chambers changed from the control treatment, as the average dimensions of the air chambers were 87.5 micrometers in length 101.125 micrometers in width. The same was the case with the vascular region, as it was noted that the thickness of the vascular region was reduced compared to the control treatment, with an average of 492.5 micrometers.

In the plants treated with a concentration of 0.4% of crude oil, it was concluded that the shape of the stem was also irregular, as the shape seemed to elongate more than the concentration of 0.3%. And the average thickness was 1895  $\mu\text{m}$ , while the thickness of the cortex was at a rate of 616.6  $\mu\text{m}$ . Also, it was indicated that some of its cells were broken. As for the dimensions of the air chambers, a fall was seen in their average dimensions, reaching (108.5)  $\mu\text{m}$  in length, and widening was found in some directions by the formation of side gaps. As for the width of the air chambers, its average was 238  $\mu\text{m}$ . The number of air chambers was reduced to 4, while the average thickness of the vascular region was 562.5  $\mu\text{m}$ .

As for the treatment with a concentration of 0.5%, which is the highest concentration used in the current study, the shape of the stem changed to be oval compared to the control treatment. The average diameter of the stem was 1725  $\mu\text{m}$ . Also, the size of the air chambers, as their average length reached 111.66  $\mu\text{m}$  and their average width reached 121.66  $\mu\text{m}$ , while the number of air chambers fell as it reached (7) aerenchyma and the disappearance of some of them, as it was seen that the thickness of the vascular area reached 483.33  $\mu\text{m}$ .

The present study disclosed the effect of different concentrations of crude oil on aquatic plants. This effect varies according to the intensity of the concentration, the type of plant, and the period of exposure to pollutants. This is consistent with the results of a study by Olanont *et al.* (2021).

When *Ceratophyllum* plant was exposed to crude oil at a concentration of 0.2%, a clear change in the shape of the stem appeared in response to these pollutants. At the beginning of exposure, the toxic components of crude oil and the size of the air chambers seemed to change gradually.

However, at the concentration of 0.3% and 0.4%, the plant began to adapt to these harsh conditions by collecting pollutants inside the parenchymal cells. Because their large gaps characterize the parenchymal cells, and the plant, in response to this toxicity, leads to the storage of air inside the air chambers. Therefore, their size begins to increase once again, however, at a high concentration, which caused poisoning of living cells, especially cells of the epidermis and cortex, due to the toxicity of the heavy elements that make up the crude oil, which leads to fading and possibly death of cells the death of the plant.

The findings of the present study are consistent with results of a study carried out by Olanont *et al.* in 2021, Olanont *et al.* in 2018, and Punwong *et al.* in 2017 that oil pollutants affected the anatomical characteristics, especially the ones of the epidermis and cortex represented by parenchymal cells, causing their reduction and dissolution of their parts. As shown it is disclosed that the species *M. Spicatm* is more resistant and stable than *C. demersum*.

Until reaching the concentration of 0.5%, the change in the thickness of the stem, the size of the air chambers, and the vascular area became clear. At the same time, the type *C. demersum* was more sensitive to the concentration, starting from 0.3% to the

concentration 0.5%. It may be due to the genetic characteristics of each plant and its tolerance. The oil droplets accumulate inside the parenchymal cells after being absorbed by the epidermis, as the parenchymal cells are often specialized for storage, followed by the epidermal cells. This makes parenchymal cells more vulnerable to damage and change in shape and size and then affects the air chambers and the shape of the stem (Omosun *et al.*, 2009).

Even though the anatomical characteristics are more stable for the stress conditions to which the plant is exposed, the length of the exposure period and the increased concentration of pollutants have a role in the effect, especially the general shape of the organ, the size of the parenchyma tissue and the shape of its cells. The plant is exposed to a decrease in size, and will affect the epidermis cells. This result is consistent with results in a study conducted by Loesani *et al.* in 2014, which made it clear that the anatomical nutritional characteristics of the *Vicia ervilia* plant when exposed to oil strain caused cracks in the epidermis vascular bundles.

In a study carried out by Olanont *et al.* in 2020, it was arrived in that mobile plants are more stable and tolerant of pollutants than fixed plants. Since the studied plants are unstable, they were more tolerant and persistent until they got a concentration of 0.5%.

Findings of the current study showed the effect of the internal anatomical characteristics of the stems in the studied plants, such as a large reduction in the vascular area, a reduction in the diameter of the stem, a decrease in the thickness of the crust area, a decrease in the size of the air chambers, and the decay of some of them in the presence of a high concentration of crude oil, thereby increasing the oil concentration leads to a gradual contraction of the plant. So, the greater the exposed oil, the longer the

period. This is due to the filling of the cells that make up the stem with hydrocarbon molecules that accumulate, closing the air chambers and preventing the natural respiration and gas exchange processes. This leads to the gradual suffocation of the plant, its decay, and later to its death. This finding is consistent with a study carried out by Baker (1970).

Figure 5 disclosed that the *Ceratophyllum* treated with a concentration of 0.2% of crude oil suffered some slight bends or zigzags in the stem and the beginning of the accumulation of polluted oil particles in the epidermis cells down to the vascular tissue. It was also found that there was a decrease in the thickness of the stem area and the size of the air chambers. Moreover, there was a gradual fall in the Vascular area because of the toxicity of the heavy elements that compose crude oil and thus affect the process of transferring ions and electrons necessary in respiration and photosynthesis. Besides, the energy falls in the plant. The present study's findings agree with investigations conducted by Baruah *et al.* (2014) and Lopes *et al.* (2009).

The current study brought to light that the first and second concentrations of crude oil (0.2%, 0.3%) caused a slight fall in the volume of the air chambers. However, the high concentrations led to a shrinkage in the volume of the air tissue (aerenchyma) and finally caused the disappearance of some chambers.

The plant treated with a concentration of 0.4%, the cross-section of the stem, there was

a change in the shape of the stem from circular to zigzag elongated from one side, which leads to a fall in the diameter of the stem by an average of 1895  $\mu\text{m}$  and a fall in the thickness of the cortex area. It began to fall gradually compared to the control treatment. Hydrocarbon molecules lead to their slow shrinkage, relying on exposure time. The epidermis and the entire cortex and a reduction in the size of the cells with an increase in concentration were seen. This is due to the direct effect of the toxicity of the constituent materials of crude oil. The impact may be gradual due to the physical damage from a gas exchange (Mostafa *et al.*, 2021; Lopes *et al.*, 2009).

It was clear that the plant *C. demersum*, when being exposed to a concentration of 0.5% of crude oil, led to the disappearance of some air gaps and the occurrence of deformation in the shape of the stem and a contraction in the vascular area and a fall in the cortex area as the distribution of hydrocarbon molecules began to reach the vascular area. The Vascular area seemed to shrink until cells and gaps are filled gradually, and cell membranes were damaged through the penetration of hydrocarbon molecules, causing leakage of pollutants into the contents of the cells and the reduction of photosynthesis rates and reduction in the length and diameter of the vascular tissues. This is because the crude oil is absorbed from the roots, stems, and leaves. This is consistent with the findings of two studies conducted by Punwong *et al.* (2017) and Maranhão *et al.* (2009).

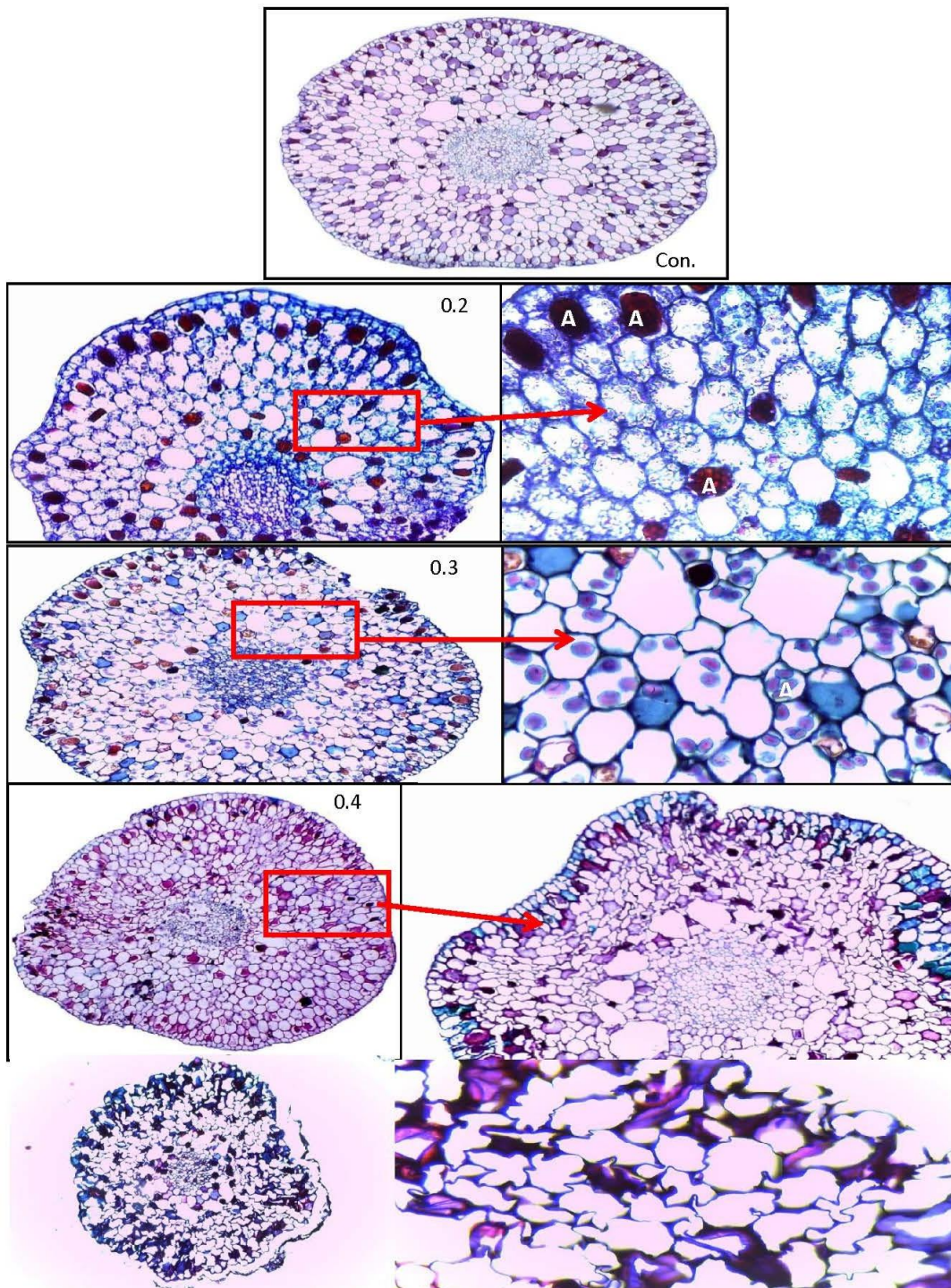


Figure 5. Anatomical alterations in the stem of *Ceratophyllum demersum* under different crude oil concentrations.

(A) Hydrocarbon accumulation within parenchyma cells; (Ar) aerenchyma showing structural reduction and deformation.

***Myriophyllum spicatum*****Control Plants**

As shown in figure 6, stems of the plants in the control treatment appeared to have a round shape, of a hard type, surrounded by a thin layer of cuticle covering one layer of the epidermis. The epidermis is followed by the cortex consisting of three areas. The outer cortex consists of three layers, and the middle cortex consists of parenchymal tissue that contains air spaces and the inner cortex that surrounds the vascular cylinder. The vascular bundle was of the central type, as the bark surrounded the wood, and the central bundles were called xylem. Its thickness averaged 35)  $\mu\text{m}$ , where there was no crushing, and it was noted that there was a clear regularity in the air chambers, where the average length of the air chambers was 260  $\mu\text{m}$ , and the average width was 284  $\mu\text{m}$ . In contrast, the vascular area had a rate not exceeding (451)  $\mu\text{m}$ . the vascular area comprises wood, cortex, and wood with few vessels and many vascular tissues.

**Plants Treated with Different Concentrations of Crude Oil**

It was seen in plants exposed to 0.2% of crude oil that there was a beginning of meandering in the transverse shape of the stem and a small increase in the average thickness of the stem diameter, which amounted to 2025  $\mu\text{m}$ . Also, there was a small increase in the average thickness of the cortex, which recorded an average of 360  $\mu\text{m}$ , compared with the control treatment, which amounted to 353  $\mu\text{m}$ . Besides, there was an increase in the average length and width of the air chambers, which amounted to 371.25 in length and 452.5  $\mu\text{m}$  in width. The center of the stem covers the vascular area, with an average thickness of 587.5  $\mu\text{m}$ . Figure 2.

As far as plants treated with a concentration of 0.3% of crude oil are concerned, a gradual increase in the diameter of the stem was

observed, and its shape changed from circular to the oval at a rate of (2560)  $\mu\text{m}$ . While the thickness of the cortex fell and its thickness reached (313.33)  $\mu\text{m}$ . Also, it was noted there was an increase in the size and elongation of the air chambers. Its average length came closer to (766.66)  $\mu\text{m}$ , and the air chambers increased in width as their average width reached (410)  $\mu\text{m}$ , with the size of the vascular region increasing, along with an average thickness of (881.66)  $\mu\text{m}$ .

At a time when the plant is treated with a concentration of 0.4% of crude oil, a noticeable deformation occurred in shape, as the shape of the stem changed to elongated for a lot of zigzags, where the average diameter of the stem in the control treatment was 1062.22  $\mu\text{m}$ . In the concentration of 0.4%, the thickness of the stem decreased, reaching (1100)  $\mu\text{m}$ , which is a significant decline, which led to the occurrence of a zigzag shape and the beginning of the reduction in one of the sides, while the average thickness of the cortex increased in other areas, reaching 448.75  $\mu\text{m}$ . Besides, there was a slight increase in the size of the air chambers, where the average length was 757.5  $\mu\text{m}$  and 307.5  $\mu\text{m}$  width, while the vascular area approached an average thickness of (862.5)  $\mu\text{m}$ .

The results disclosed that the treatment with a concentration of 0.5% of crude oil changed the shape of the stem, as it became irregular, zigzag, and shrunken on one side rather than the other. Also, some air chambers' disappearance led to a significant reduction in the size of the air chambers, as the average diameter reached 1620  $\mu\text{m}$ . While the thickness of the cortex reduced significantly as it approached 2937.5  $\mu\text{m}$ , and the size of the air chambers reduced considerably as it reached a length of 400  $\mu\text{m}$  and a width of 187.  $\mu\text{m}$ . Moreover, the number of air chambers fell to 6, which led to the reduction of the vascular area from the same direction as the spread of polluted oil particles, and its

thickness averaged 445  $\mu\text{m}$ . As the figures indicated, the shape of the parenchymal cells changed from round to irregular zigzag in shape.

Figure 6 sheds light on comparing the visceral airway cells. In the control sample, the air chambers were normal and of homogeneous size and in the low concentrations of 0.2% and 0.3%, where no significant effect on the volume of the air chambers was noted. As for the concentration of 0.4%, the small size of the air chambers and the folding of the walls separating each other were seen. This is due to the contraction that occurred to the cells of the air chamber walls as a result of exposure to the pollutant, which leads to an imbalance in the osmotic pressure of the internal medium of the cell compared to the surrounding medium. Therefore, the cells shrink, causing folding, or the wall of the air chambers is dented, which affects their size. Consequently, the amount of gases inside these cells drops, and leads to physiological decline and eventual plant mortality, and many of the air chambers in the tissues of the *Myriophyllum spicatum* plant disappeared.

Second, when the plant was treated with 0.2% of crude oil, the shape appeared with simple bends in the shape of the cross-section of the stem, the diameter of the stem increased, an increase in the thickness of the cortex and the area of the air chambers. Then was an increase in the thickness of the vascular area, which means an expansion in the cross section of all its contents due to the entry of the crude oil into the cells, bringing about an increase in its size. The justification for the increase could be due to a reaction by the plant to resist the uptake of the pollutant and the irregular entry of hydrocarbon molecules, which led to stimulating cells filled with hydrocarbon molecules to widen the cortex area. The toxic crude oil starts to accumulate in the parenchymal cells and the interstitial spaces, thus reducing the distance

of these cells. This is consistent with the findings of two studies carried out by Baker (1970) and Punwong *et al.* (2017).

Third, it is disclosed as a result of treatment of plants with a concentration of 0.3% of crude oil there was a clear change in the shape of the stem and it tends to almost become oval in shape at a rate of (2560)  $\mu\text{m}$ . The cortex cells began to reduce in size due to the prevention of the transfer of metal ions to the tissues or to the process of photosynthesis and the occurrence of deformation in the epidermis area and vascular bundles. This deformation is more significant because the crude oil concentration is higher. This is consistent with findings resulting from a study conducted by Gomes *et al.* (2011).

Fourth, as for the plant treated with a concentration of 0.4% of crude oil, there were clear differences in the cross-section. An apparent change was observed in the shape of the stem, as it changed from a circular shape to an elongated oval. Also, a contraction occurred on the elongated side of the section. It expanded on the other side, due to the slow and irregular entry of hydrocarbon molecules, where the more viscous the oil is. The higher the concentration, the slower and less regular the passage of the hydrocarbon molecules is, as it passes through the cell walls and membranes. However, because of the presence of quantities of water and as the studied plant is aqueous, it may hinder its passage, but the force of gravity, environmental factors, and tissue movement lead to the oil merging with the oil stains originally available inside the plant. This causes changes in the vascular area, an increase in cracking in the cortex area, an irregularity in its size, and an increase in the difference in the cross section. This is consistent with findings of two studies carried out by Baker (1970) and Lorestani *et al.* (2014).

Finally, as far as the 0.5% concentration is concerned, the shrinking cross section was seen on one side due to the disappearance of some air chambers. This phenomenon led to an increase in the diameter of the stem and the thickness of the cortex and a fall in the thickness of the vascular area because the irregularly distributed hydrocarbon molecules brought about a structural deformation in the section, i.e. a deformation in the vascular area and a rupture in some of the cytoplasmic membranes in the epidermis and cortex. This leads to damage to the

plasma membrane and its permeability and impregnation of substances constituting oil. Then spaces between the cells are filled due to the absorption of quantities of oil, which leads to the accumulation of the materials that make up the oil in holes, where they begin to clump with each other, which led to cellular deformation. Therefore, the plant can be used as a biological indicator for contamination. This finding is consistent with results obtained in two studies conducted by Akapo (2011) and Baker (1970).

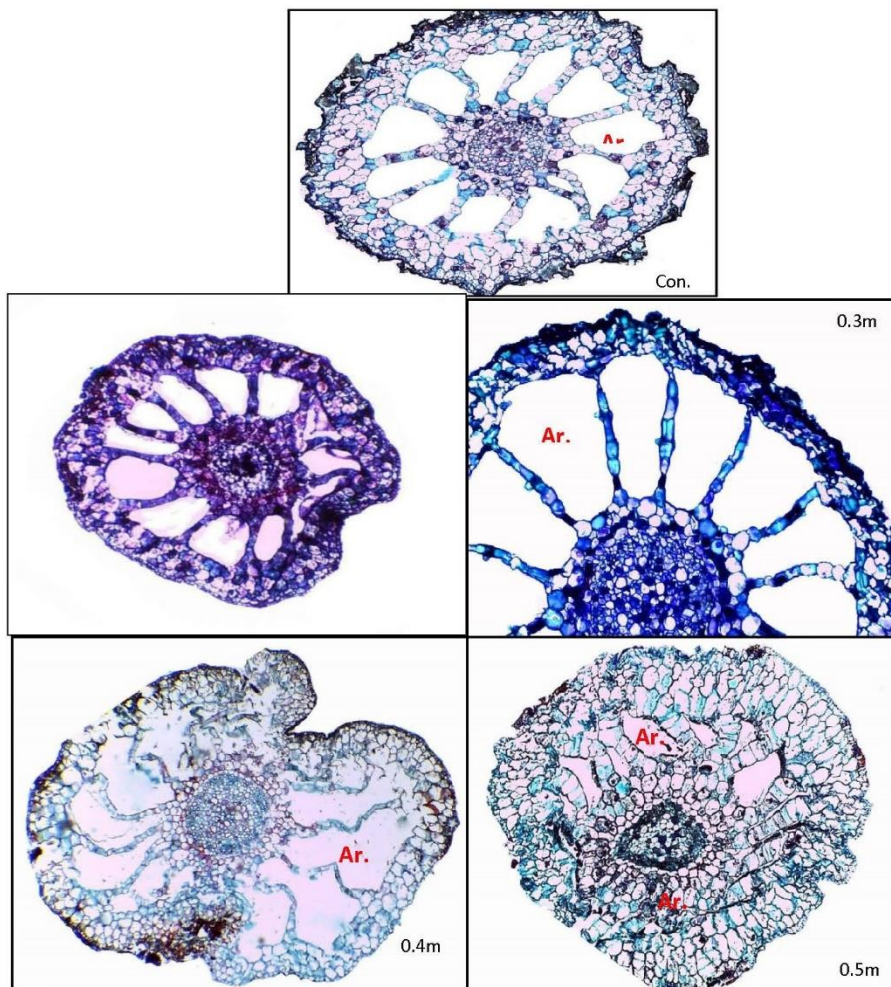


Figure 6. Anatomical alterations in the stem of *Myriophyllum spicatum* under different crude oil concentrations.

(A) Accumulation of hydrocarbon compounds within parenchyma cells; (Ar) aerenchyma tissue showing structural modification.

**Conclusion:**

Crude oil contamination decreased chlorophyll and protein concentration in both species of aquatic plants, viz., *Ceratophyllum demersum* and *Myriophyllum spicatum*, in a dose-dependent way, proving damage of photosynthesis and metabolism of aquatic plants.

Anatomy of both the species showed severe damage and reduction in aerenchyma development, reduction of cortical thickness, shrinkage of vascular tissue, etc., at all contamination level and there was more damage at the higher doses of oil (0.4, 0.5%). Degradation was observed as due to hydrocarbon-induced oxidative stress and membrane damages, which could lead to the damage to crucial physiological processes. Two species studied differed in relative tolerability toward the hydrocarbon contamination and one species was much more tolerating to this effect under the higher range of crude oil concentration.

**References:**

- Akapo, A. A. R., Omidiji, S. O., & Otitoloju, A. A. (2011). Morphological and anatomical effects of crude oil on *Pistia stratiotes*. *The Environmentalist*, 31(3), 288–298.
- Aljamali, N. M., & Salih, N. S. (2021). Review on chemical separation of crude oil and analysis of its components. *Journal of Petroleum Engineering & Technology*, 11(2), 35–49.
- Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1–15.
- Al-Moaikal, R. M. S., Shukry, W. M., Azzoz, M. M., & Al-Hawas, G. H. S. (2012). Effect of crude oil on germination, growth and seed protein profile of *jojoba* (*Simmondsia chinensis*). *Plant Science Journal*, 1, 20–35.
- Al-Edany, T. Y., & Al-Mayah, A. A. (2001). Pollen morphology and taxonomy of Convolvulaceae. *Basrah Journal of Agricultural Sciences*, 13(2), 211–299.
- Baker, J. M. (1970). The effects of oils on plants. *Environmental Pollution*, 1(1), 27–44.
- Bayat, Z., Hassanshahian, M., & Hesni, M. A. (2016). Symbiotic crude oil-degrading bacteria in *Mactra stultorum* from the Persian Gulf. *Marine Pollution Bulletin*, 105(1), 120–124.
- Baruah, P., Saikia, R. R., Baruah, P. P., & Deka, S. (2014). Effect of crude oil contamination on chlorophyll content and morpho-anatomy of *Cyperus brevifolius*. *Environmental Science and Pollution Research*, 21(21), 12530–12538.
- Bhatia, S. C. (2009). *Environmental pollution and control in chemical process industries* (2nd ed.). Khanna Publishers.
- Gomes, M. P., Marques, T. C. L. L., Melo, S., & Soares, A. M. (2011). Ecophysiological and anatomical changes due to heavy metal accumulation in *Brachiaria decumbens*. *Scientia Agricola*, 68, 566–573.
- Hinojosa, M. B., Carreira, J. A., Ruiz, R. G., & Dick, R. P. (2004). Soil moisture pretreatment effects on enzyme activities in contaminated soils. *Soil Biology and Biochemistry*, 36, 1559–1568.
- Izzati, M. (2015). Salt tolerance of several aquatic plants. *Proceedings of the International Conference on Global Resource Conservation*, 6, 1–10.
- Lopes, A., Rosa-Osman, S. M., & Piedade, M. T. F. (2009). Effects of crude oil on aquatic macrophytes from Amazon

- floodplains. *Hydrobiologia*, 636(1), 295–305.
- Lorestani, B., Kolahchi, N., Ghasemi, M., & Cheraghi, M. (2014). Changes in germination, growth, and anatomy of *Vicia ervilia* in response to light crude oil stress. *Journal of Chemical Health Risks*, 4(1), 45–52.
- Maranho, L. T., Dziedzic, M., Muñiz, G. I. B., Kuniyoshi, Y. S., & Galvão, F. (2009). Effects of petroleum pollution on plant tracheids. *Brazilian Journal of Biology*, 69(2), 263–269.
- Ma, Z., Zhang, M., Xiao, R., Cui, Y., & Yu, F. (2017). Soil microbial community changes in coastal wetlands. *Geoderma*, 289, 124–134.
- Mostafa, A. A., Hafez, R. M., Hegazy, A. K., Fattah, A. M., Mohamed, N. H., Mustafa, Y. M., & Azab, E. (2021). Structural and functional responses of *Azolla pinnata* to crude oil pollution. *Sustainability*, 13(4), 2142.
- Omosun, G., Edeoga, H. O., & Markson, A. A. (2009). Anatomical changes due to crude oil pollution in *Mucuna* species. *Recent Research in Science and Technology*, 1, 264–269.
- Olaranont, Y., Stewart, A. B., & Traiperm, P. (2018). Physiological and anatomical responses of beach grass to crude oil pollution. *Environmental Science and Pollution Research*, 25(28), 28075–28085.
- Olaranont, Y., Stewart, A. B., & Traiperm, P. (2021). Effects of crude oil on plant growth and leaf anatomy. *International Journal of Phytoremediation*, 23(2), 162–170.
- Olubodun, S. O., & Eriyamremu, G. E. (2015). Effects of different crude oil fractions on total chlorophyll, glucose, and starch content of *Zea mays*. *Journal of the Nigerian Society for Experimental Biology*, 15(1), 11–16.
- Punwong, P., Juprasong, Y., & Traiperm, P. (2017). Effects of oil spill on leaf anatomy of *Terminalia catappa*. *Environmental Science and Pollution Research*, 24(27), 21821–21828.
- Tamire, G., & Mengistou, S. (2013). Macrophyte diversity in Lake Ziway, Ethiopia. *African Journal of Ecology*, 51(1), 66–77.
- Page, A. L., Miller, R. H., & Keeney, D. R. (1982). *Methods of soil analysis: Part 2* (2nd ed.). American Society of Agronomy.
- United Nations Environment Programme. (2019). *Global environment outlook – GEO-6: Healthy planet, healthy people*. Cambridge University Press.

## تأثير تلوث النفط على بعض الخصائص البيوكيميائية والتشريحية لنباتي *Ceratophyllum demersum* و *Myriophyllum spicatum*

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### المستخلص

تهدف هذه الدراسة إلى تقييم تأثير تراكيز مختلفة من النفط الخام على بعض الخصائص البيوكيميائية والتشريحية لنوعين من النباتات المائية المغمورة هما *Ceratophyllum demersum* L. و *Myriophyllum spicatum* L. شملت المعاملات التجريبية مجموعة سيطرة (0% نفط خام) ومعاملات أخرى أضيف إليها النفط الخام بتراكيز 0.2% و 0.3% و 0.4% و 0.5%. أظهرت النتائج حدوث انخفاض معنوي في محتوى الكلوروفيل الكلي في نبات *C. demersum* عند تركيز 0.5% حيث بلغ (1.0424 ملغم/لتر)، وكذلك في نبات *M. spicatum* عند نفس التركيز حيث بلغ (0.3552 ملغم/لتر). كما كشفت الدراسة التشريحية للسيفان عن تأثيرات سلبية لجميع تراكيز النفط الخام المختبرة، وبشكل أوضح عند التراكيز العالية (0.4% و 0.5%).

في المقابل، كانت التراكيز المنخفضة (0.2% و 0.3%) أقل ضرراً مقارنة بالتراكيز الأعلى، مما يشير إلى وجود قدرة نسبية لدى هذه النباتات على تحمل مستويات متزايدة من التلوث النفطي. وقد تمثل ذلك في انخفاض حجم خلايا النسيج الهوائي (aerenchyma)، مع اختفاء بعضها بشكل كامل في بعض الحالات. وبالمقارنة بين النوعين، أظهر نبات *C. demersum* قدرة تحمل أعلى للتراكيز المرتفعة من النفط الخام مقارنة بنبات *M. spicatum*. كما لوحظ في كلا النوعين انخفاض في قطر الساق، وتناقص في سمك القشرة، بالإضافة إلى انخفاض في حجم الأنسجة الوعائية.

الكلمات المفتاحية: تلوث النفط الخام، النباتات المائية، الاستجابات البيوكيميائية والتشريحية