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Changes in the anatomical characteristics of Avicennia marina (Forssk.) Vierh leaves exposed to petroleum hydrocarbon pollution

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Abstract

The current study showed the effect of the anatomical characteristics of Avicennia marina (Forssk.) Vierh leaves exposed to different concentrations of petroleum hydrocarbon compounds. These trees are important plants that have been cultivated in our time on the shores of the Shatt al-Arab in Basra Governorate, contributing to reducing the risks of environmental changes and creating a coastal environment with high biodiversity. The cross-section of the leaf was studied, and the highest average thickness was recorded at concentrations (2.5 and 10) ppm, reaching 425 and 423µm respectively, while the lowest concentration was recorded at 255µm in the control plants. The change in the thickness of both the upper epidermis and the lower epidermis was also observed, with the upper epidermis recording a greater thickness than the lower epidermis in all samples, and a clear difference between them is evident, as the highest average thickness of the upper epidermis ranged from 181.4µm at 2.5ppm concentration compared to the control samples, which reached 122µm, while the lowest average thickness was 128µm at 7.5ppm concentration. Changes in palisade layer thickness were also observed in the samples exposed to different concentrations of petroleum hydrocarbons. The columns show a clear variation in palisade layer thickness between the different samples compared to the control samples. The midrib thickness of A. marina leaves exposed to the same concentrations was also affected. Furthermore, the upper and lower collenchyma layers of the leaf exhibited significant effects. The collenchyma thickness in the lower layer was found to be greater than the upper layer in all samples.

Keywords: Avicennia marina, anatomy characteristic, Hydrocarbons, Basrah.

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Introduction

Coastal *Avicennia marina* (Forssk.) Vierh ecosystems are among the most diverse and productive ecosystems in tropical and subtropical regions. However, they face significant environmental threats due to increasing human activities and oil spills. Because of the high toxicity of petroleum hydrocarbons and their difficulty in decomposing naturally in coastal sediments, they pose a constant threat to coastal biodiversity. Numerous studies have emerged as a green, sustainable, and low-cost alternative for cleaning up oil-contaminated environments. *A. marina* s play a pivotal role in this technology thanks to their exceptional ability to survive and grow in harsh environments characterized by high salinity and low oxygen levels. This enables them to act as natural reservoirs and catalysts for hydrocarbon biodegradation processes in intertidal zones. However, the efficiency of phytoremediation using plants alone remains limited and is affected by the concentration of petroleum pollutants and their physical and chemical behavior. (Aryal, 2024).

Youssef (2002) evaluated stomatal behavior, growth rate, and polycyclic aromatic hydrocarbon (PAH) accumulation in *Avicennia marina* (Forssk.) Vierh. seedlings treated with the water-soluble fraction (WSF) of Abu Dhabi

Arabian Light crude oil, either by foliar spraying or soil application. Irregular stomatal behavior and impaired transpiration were observed during the first 24 hours. Tricyclic aromatic hydrocarbons accumulated most in the tissues of the treated plants. A linear relationship was observed between the dose applied to the plants and the amount of PAHs accumulated in the tissues.

Hidayati *et al*. found (2018) showed that *A. marina* s, such as the species *A. marina* and *Rhizophora* mangle, have an exceptional ability to treat pollution with petroleum hydrocarbons in coastal environments through the mechanisms of rhizodegradation and biosorption within their tissues, and this effectiveness increases significantly when combined with fertilization or the addition of auxiliary bacteria.

Al-Zewar *et al*., (2023) studied the growth indicators of *A. marina* trees planted in the intertidal zone of Khor Al-Zubair oil port. They observed that there were high growth indicators for the *A. marina* trees, including increased plant height, number of lateral branches, total number of leaves, and total chlorophyll content during the nine-month monitoring period.

A. marina species differ in their physiological response levels and their ability to adapt and survive under increased oil stress. In an experiment conducted by (Tang, 2024) that lasted for six months to estimate the impact of marine diesel pollution on *A. marina* trees red seedlings (*Rhizophora mangle*) showed physiological tolerance and survival rates at much higher rates compared to gray *A. marina* seedlings (*Avicennia schaueriana*), which suffered high rates of deterioration, reaching 100% in the absence of helpful bacteria, thus proving the variation in sensitivity and defensive ability between species.

In an applied study evaluating the efficiency of *A. marina* -supported phytoremediation in crude oil-contaminated soil from the Zubair oil field in Iraq, the addition of nitrogen, phosphate, and potassium (NPK) fertilizers was found to make a significant and statistically significant difference in the rate and efficiency of decomposition. Soil enriched with NPK fertilizer recorded the highest rate of biodegradation at 77%, while the decomposition efficiency in unfertilized soil (control group) dropped to a minimum of 51% (Al-Shemary, 2026).

Materials and Methods

The current study was conducted at the Marine Science Center - University of Basra during the period from September 2024 to February 2025. *A. marina* tree seeds were brought from the Islamic Republic of Iran. The seeds were washed thoroughly with distilled water and then left for 24 hours in water to remove the outer shell of the seed. (Al-Zewar *et al*., 2023; Al-Mayah and Al-Asadi, 2023)

I took 50 seeds of the same size and they were grown in bags made of cloth (an environmentally friendly material) of a size of one liter containing a mixture of soil. They were placed in a basin containing distilled water and left for three months for the plants to grow. After the seedlings reached a height of 25-30 cm, I took eighteen seedlings of the same height (30 cm) at a rate of three replicates per treatment (0, 1.5, 2.5, 5, 7.5, 10).

Preparation of Oil Concentrations

Crude oil was obtained from the Basrah Oil Company. Five concentrations were prepared: (1.5 , 2.5 , 5 , 7.5 , and 10)ppm. These concentrations were prepared according to the dilution law, using the required amount of oil and diluting the concentration with distilled water. A control treatment, consisting solely of distilled water, was also used.

The seedlings (*Avicennia marina)* were irrigated with the prepared concentrations every two days for three months (from December 2018 to February 2016). The plants were monitored until the end of the exposure period. Afterward, mature leaves were collected, and the following procedures were performed:

The rotary microscope method was used, based on the model of Johanson (1968), with modifications:

1.The soft parts (leaves) of *Avicennia marina* plants were fixed and placed in a fixative

solution of formalin, acetic acid, and ethyl alcohol (FAA) for 24 hours.
2.The plant parts were washed with 70% ethyl alcohol to remove any traces of the fixative solution. 3. The soft tissues were then passed through a series of ascending ethyl alcohols (70%, 80%, 95%, and 100%) for two hours at each concentration. Next, the samples were passed through a series of absolute ethyl alcohol to xylene mixtures in ratios of 3:1, 1:1, and 1:3 for 45 minutes at each concentration. Afterward, the samples were placed in pure xylene for half an hour, then in a mixture of xylene and molten paraffin wax in an oven at 60°C for three hours. Finally, they were placed in pure paraffin wax and left overnight at the same oven temperature.
4.Pure paraffin wax was then prepared and poured into metal cubes. The plant specimens were placed in the cubes, which were labeled with the specimen numbers under study.
5. The cubes were left to cool in the refrigerator overnight, after which they were ready for dissection using a rotary microtome. 6. The specimens were cut to a thickness of 10–25µm and then flattened by placing the sections on the surface of water in a 45°C water bath. They were then placed on glass slides and incubated on a hot plate at 30–50°C for 30 minutes.
6. The specimens were then placed in pure xylene overnight. The following day, they were passed through a descending series of ethyl alcohols (70%, 90%, 95%, and 100%) for 10 minutes at each concentration.
7. They were then stained with safranin red, prepared by dissolving 1 gram of the dye in 100 ml of distilled water for 15 minutes. Finally, they were passed through an ascending series of ethyl alcohols (70%, 90%, 95%, and 100%) to remove any remaining dye.
8. After washing, the slides were placed in Fast Green stain prepared by dissolving 1 gram of the stain in 100 ml of absolute ethyl alcohol for 5 seconds.
9. The slides were then washed in absolute ethyl alcohol and subsequently passed through purified xylene for 5 minutes (twice). After air drying, a few drops of Canada Balsam were applied, a cover was placed over the slide, and the slide was placed on a heated plate at 60°C for one hour. The slides were then ready for examination and study. The samples were photographed using a light microscope.

Results and Discussion

Leaf Thickness

Figure (1) shows the leaf thickness rates in *Avicennia marina* trees exposed to different concentrations of petroleum hydrocarbons. The highest leaf thickness rates were recorded at concentrations (2.5 and 10) ppm, reaching 425 and 423µm respectively, while the lowest concentration of 255µm was recorded at the first concentration (control plants). The results show a difference in leaf thickness rates in the histological section of *A. marina* leaves. The thickness increased in plants exposed to high concentrations of petroleum hydrocarbons compared to control plants. This may be due to the fact that when plants are subjected to stress, they adopt several protective strategies, including increasing epidermal thickness and increasing cuticle deposition, in order to prevent the entry of pollutants into their tissues and to reduce water loss (Chaurasia *et al*., 2022; Patel *et al*., 2024). (Plate, 1)

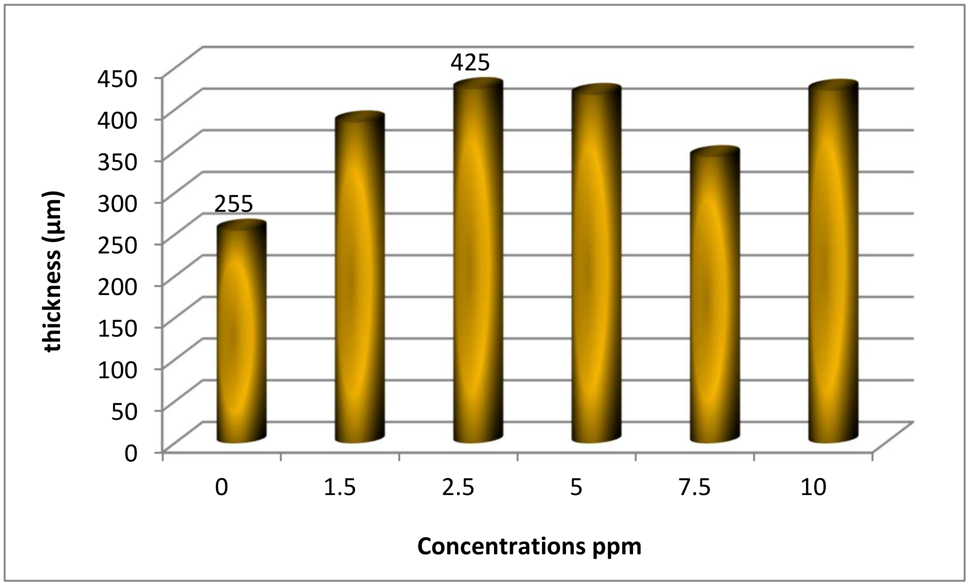


Figure (1): Average Cross-Sectional Thickness of *A. marina* Leaf Exposed to Different Hydrocarbon Concentrations

Epidermal Thickness

Figure (2) illustrates the changes in average thickness of the upper and lower epidermis of *A. marina* leaves exposed to different concentrations of petroleum hydrocarbon compounds. The upper epidermis was thicker than the lower epidermis in all samples, with a clear difference between them. The highest average thickness of the upper epidermis was 181.4µm at 2.5ppm concentration, compared to 122µm in the control samples. The lowest average thickness was 128µm at 7.5ppm concentration. The greater thickness of the upper epidermis indicates that it plays a key role in protecting the leaf from environmental factors such as intense solar radiation, high temperatures, and reducing water loss through transpiration. The lower epidermis is thinner than the upper epidermis, and this is related to the presence of stomata on its surface, which facilitates gas exchange while minimizing water loss. The anatomical differences between samples may reflect plant adaptations to the pollution stress they have been exposed to, especially at high concentrations. The thicker epidermis acts as a barrier, reducing the penetration of pollutants into internal tissues and limiting water loss, particularly in coastal *A. marina* environments. Exposure to hydrocarbons can also lead to anatomical changes in the leaf, such as increased thickness of certain cuticle layers due to the plant's stimulation of increased production of cell walls and the waxy layer. This type of anatomical change is a defensive response that helps *A. marina* plants withstand harsh environmental conditions and reduce damage from oil pollutants (Nkansah, 2025). Furthermore, the anatomical features of leaves can reflect plant adaptation to changing environmental conditions, thus influencing ecosystem functions due to their ability to regulate gas exchange and, to some extent, light absorption (He *et al*., 2018). (Plate, 1)

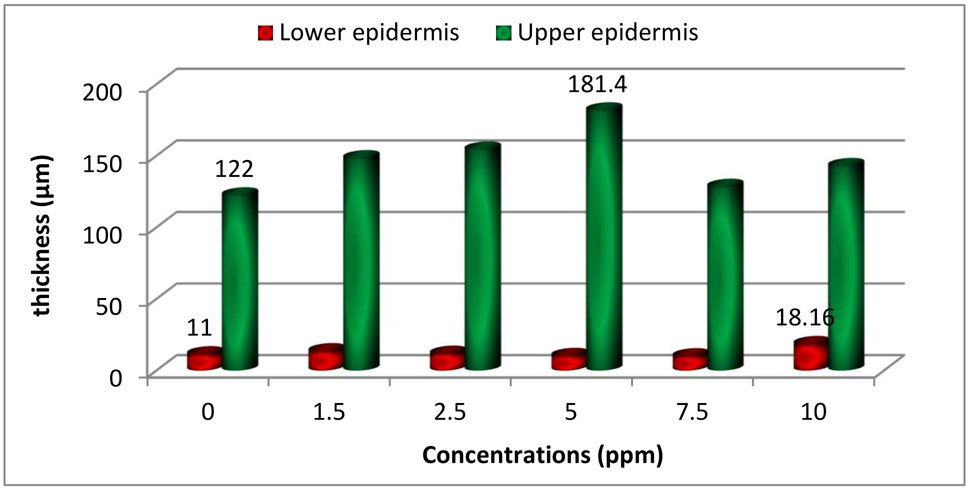


Figure (2): Average thickness of the upper and lower epidermal layers of *A. marina* leaves exposed to different concentrations of hydrocarbons

Palisade layer thickness

Figure (3) illustrates the changes in palisade layer thickness among the different samples exposed to various concentrations of petroleum hydrocarbons. The columns show a clear variation in palisade layer thickness between the different samples compared to the control samples. The highest average thickness was 128µm at 5ppm concentration, and the lowest was 88.57µm at the 7.5ppm concentration, compared to the control plants, which recorded a thickness of 72µm.

The variation in palisade layer thickness between the samples indicates differences in the leaves' ability to adapt to the pollution they are exposed to. The palisade layer is the tissue responsible for the majority of photosynthesis due to its high density of chloroplasts. Therefore, an increase in its thickness is often associated with higher photosynthetic efficiency. The large thickness recorded at the high concentration may reflect an adaptation to high light conditions or the availability of water and nutrients, leading to an increase in the number or length of palisade cells. In contrast, the marked decrease in the fifth concentration may be due to the plant being exposed to pollution stresses. (Plate, 1)

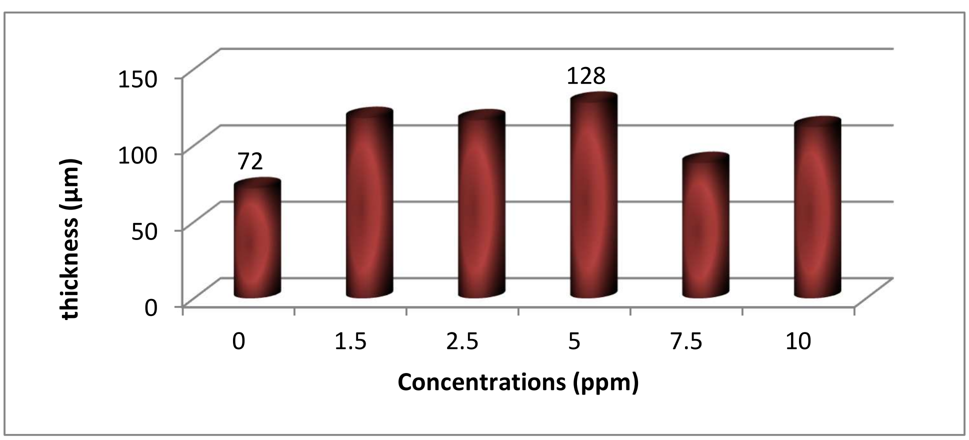


Figure (3): Average cross-sectional thickness of the palisade layer in *A. marina* leaf exposed to different concentrations of hydrocarbons

Spongy mesophyll thickness

Figure (4) shows the changes in average spongy mesophyll thickness in *A. marina* leaves exposed to different concentrations of petroleum hydrocarbons. The highest average spongy mesophyll thickness was recorded at approximately 76µm at 1.5ppm concentration, and the lowest at 40µm at 7.5ppm concentration, compared to the control samples, where the thickness was 39µm. The increase in spongy mesophyll thickness in *A. marina* leaves exposed to petroleum stress may be attributed to the fact that this layer is important for gas exchange and increasing the efficiency of photosynthesis. Furthermore, the increased thickness indicates greater development of the mesophyll tissue within the leaf, an increase in the number or size of spongy cells and air spaces, and an improvement in the diffusion of carbon dioxide within the leaf, thus increasing the efficiency of photosynthesis for the purpose of adapting the plant to petroleum stress (He *et al*., 2018). (Plate, 1)

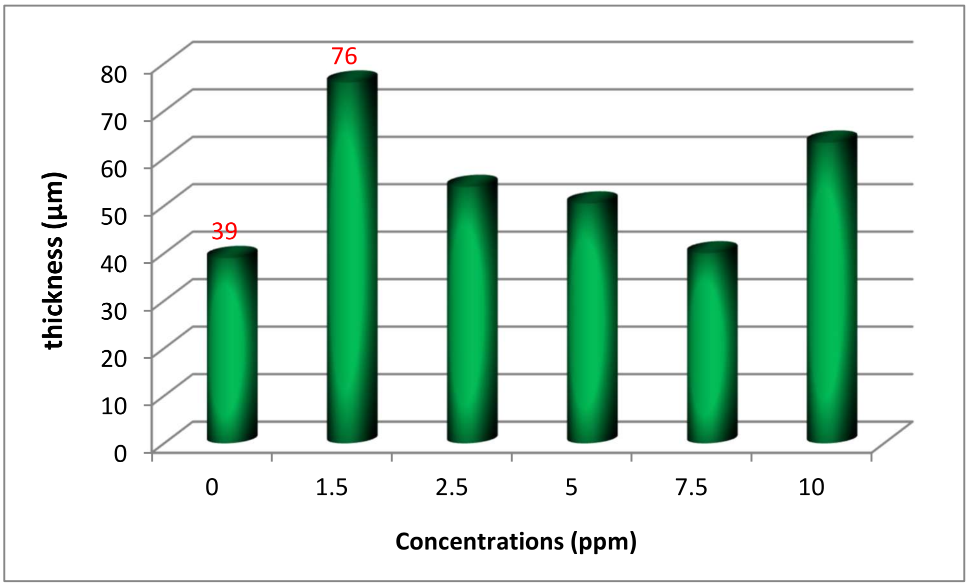


Figure (4): Average spongy mesophyll thickness of the palisade layer in *A. marina* leaf exposed to different concentrations of hydrocarbons

Medrib Thickness

Figure (5) shows the changes in average thickness of *A. marina* leaves exposed to different concentrations of petroleum hydrocarbons. The highest average thickness was recorded at 625µm at 7.5ppm concentration, and the lowest at 511.6µm at 10ppmconcentration, compared to 535µm in the control samples.

The variation in midrib thickness among the samples indicates an anatomical response of the plant to varying levels of pollution stress. The midrib is the primary component responsible for the mechanical support of the leaf and the transport of water, nutrients, and organic matter. Therefore, an increase in midrib thickness may reflect an adaptation aimed at enhancing transport efficiency and improving leaf rigidity. The increased midrib thickness in *A. marina* leaves exposed to hydrocarbon pollution may be due to an adaptive response to the stress caused by petroleum pollutants. This could lead to increased development of vascular and supporting tissues to maintain efficient water and nutrient transport and reduce the impact of pollutants on physiological processes. Conversely, a decrease in thickness at concentration 6 may indicate that the plant was subjected to greater stress or that its ability to adapt was reduced, resulting in decreased growth of vascular and supporting tissues. However, the relative similarity of the values among the samples suggests that all plants retained a similar anatomical structure, with variations reflecting the intensity of the environmental response rather than a radical change in anatomical structure. Alternatively, this may be because plants, when exposed to high pollution stress, increase leaf tissue thickness to protect themselves from the pollution. Sanchez-Landero *et al*. (2024) observed that urban trees have thicker epidermal tissue than those in rural areas, without any corresponding changes in mesophyll or leaf thickness. These differences between studies may be attributed to variations in species resistance to different types of pollution. (Plate, 1)

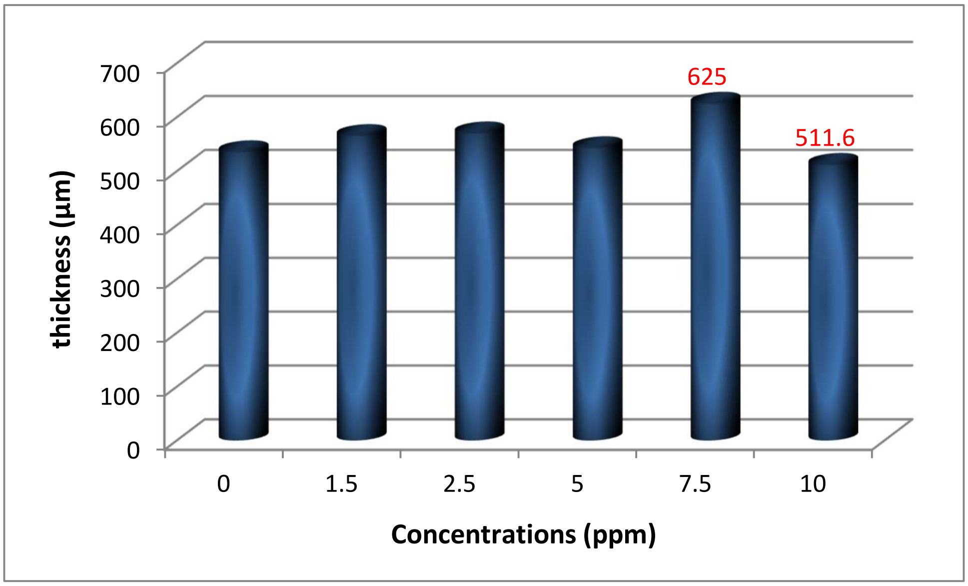


Figure (5): Average thickness of the midrib in a *A. marina* leaf exposed to different hydrocarbon concentrations.

Collenchyma tissue

Figure (6) shows the changes in the average thickness of the upper and lower collenchyma tissue layers in a *A. marina* leaf exposed to different oil concentrations. It appears that the collenchyma tissue in the lower layer is thicker than the upper layer in all samples. The highest thickness of the upper layer was 106µm at 7.5 concentration, while the highest thickness of the lower layer was 162µm at 7.5ppm concentration. The lowest thickness of the upper collenchyma tissue was 35.38µm at concentration 3, while the lowest thickness of the lower layer was 100µm at the same concentration.

The results indicate a clear increase in collenchyma thickness in the lower layer compared to the upper layer, suggesting that the lower layer plays a greater role in providing mechanical support to the leaf or plant organ. This is related to the exposure of this layer to mechanical stresses or environmental conditions that necessitate an increase in the thickness of the supporting tissue. Differences were also observed between the samples, with sample five showing the greatest thickness. This may reflect an adaptive response to different environmental conditions, such as pollution-induced stress, or it may be a defensive and adaptive mechanism. The plant strengthens its tissues to withstand stress from oil pollutants, thus helping to maintain tissue rigidity and reduce damage from toxic effects.

The reduced thickness in some samples may indicate impaired growth or a decreased ability of the plant to form supporting tissues due to the severity of the pollution. (Akcin *et al*., 2025; Nazir *et al*., 2021; Leroux, 2012). (Plate, 1)

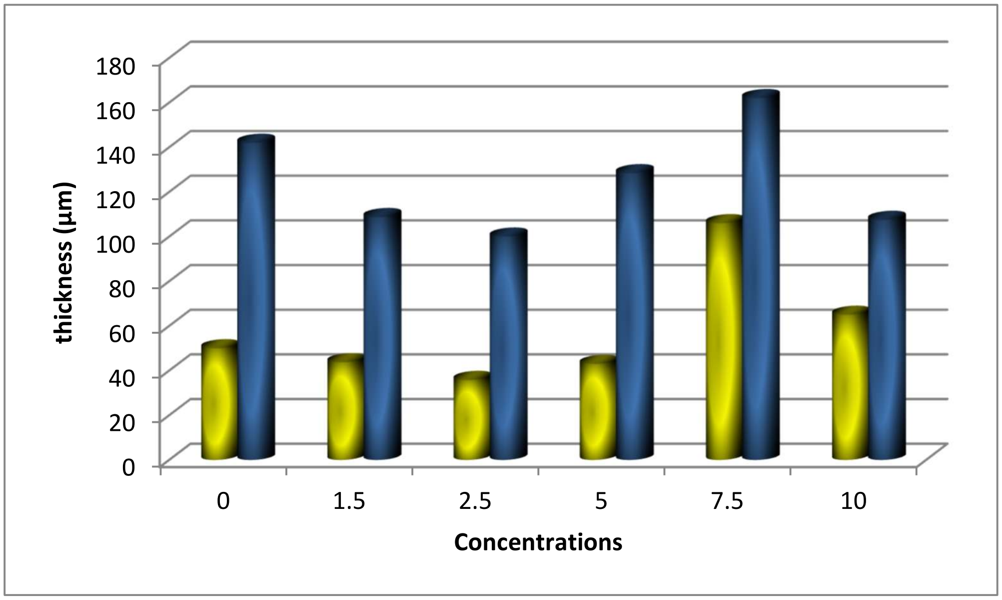
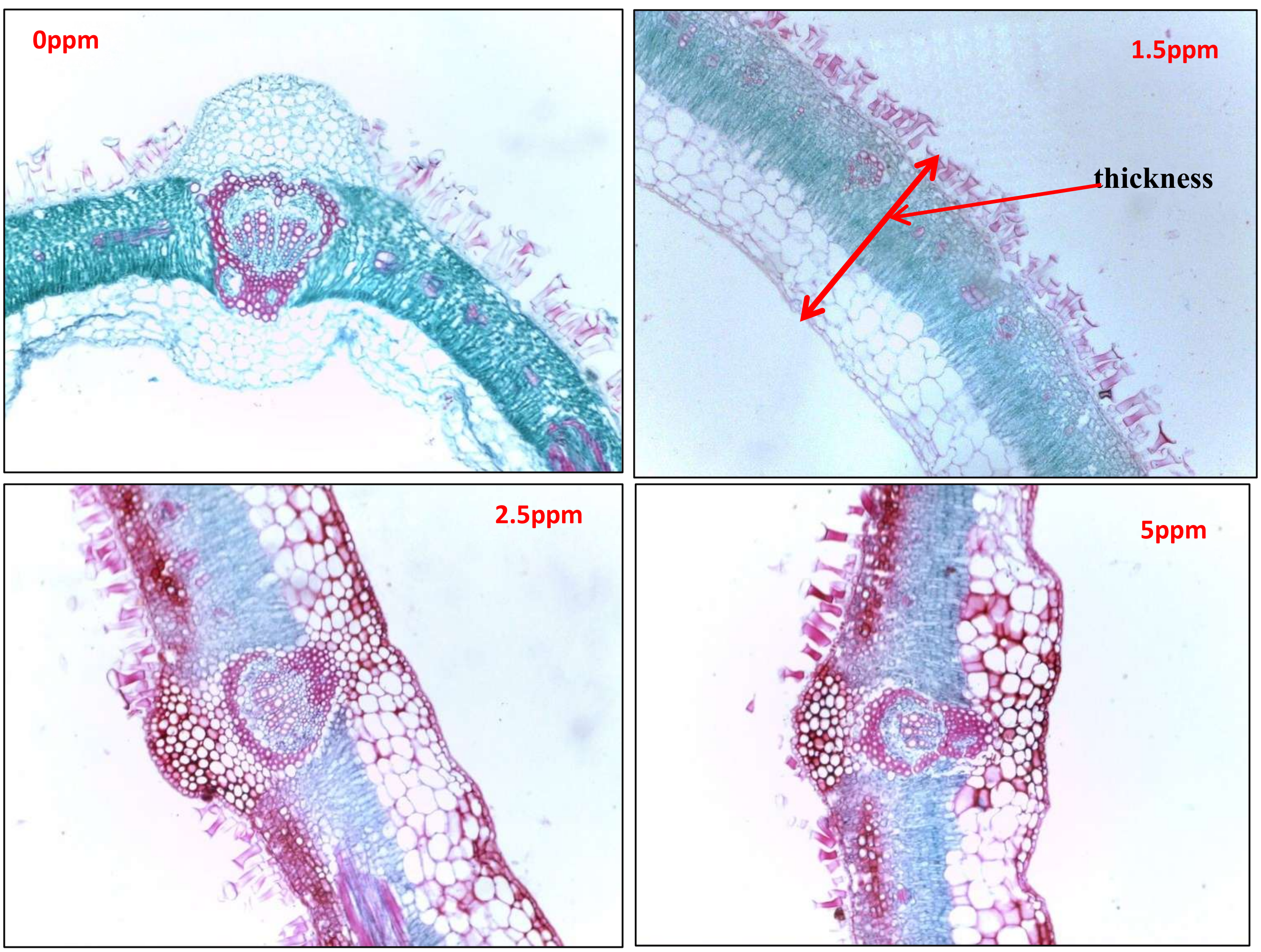


Figure (6): Average thickness of the collenchyma cell layer of a leaf in *A. marina* s exposed to different concentrations of hydrocarbons



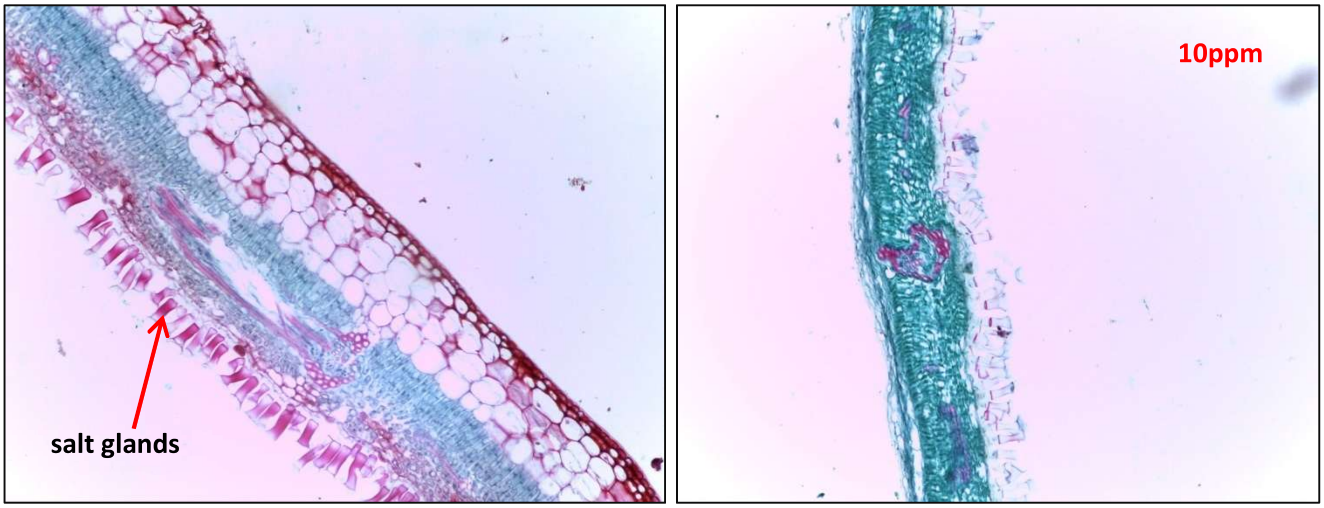


Plate (1) Changes in leaf thickness of *A. marina* exposed to different concentrations of petroleum hydrocarbons

Conclusions

We conclude from this study, which showed the effect of different concentrations of crude oil on the anatomical characteristics of *A. marina* leaves, that these trees are tolerant of concentrations, especially high ones, as their internal tissues have adopted protective strategies against this stress by thickening their cells, increasing the thickness of their walls, and shrinking their cells in order to protect them and continue their growth in the presence of high pollution stress.

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التغيرات في الصفات التشريحية لاوراق *Avicennia marina* المعرضة للتلوث بالهيدروكاربونات النفطية

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الخلاصة

بينت الدراسة الحالية تأثير الصفات التشريحية لاوراق اشجار *Avicennia marina* المعرضة لتراكيز مختلفة من المركبات الهيدروكاربونية النفطية، والتي تعد هذه الاشجار من النباتات المهمة التي استزرعت في وقتنا الحالي على سواحل شط العرب في محافظة البصرة اسهامنا لتقليل مخاطر التغيرات البيئية وصنع بيئة ساحلية ذات تنوع احيائي عالي. اذ درست كل من المقطع المستعرض للورقة والتي سجل أعلى معدل سمك لها في التركيزين (2.5 و 10) جزء بالمليون، إذ بلغا 425 و 423 ميكروميتر على التوالي، فيما سجل ادنى تركيز 255 ميكروميتر عند نباتات السيطرة، كما لوحظ التغير في سمك كل من البشرة العليا Upper epidermis والسفلى Lower epidermis لها فسجلت البشرة العليا أكثر سمكًا من البشرة السفلى في جميع العينات، ويظهر فرق واضح بينهما، اذ تراوح اعلى معدل سمك للبشرة العليا 181.4 ميكروميتر عند التركيز 2.5 جزء بالمليون مقارنة مع عينات السيطرة التي بلغت 122 مايكروميتر اما ادنى معدل لسمك كان 128 مايكروميتر عند التركيز 7.5 جزء بالمليون. كما لوحظ التغيرات في معدلات سمك الطبقة العمادية (Palisade layer thickness)المعرضة لتراكيز مختلفة من الهيدروكاربونات النفطية، اذ تظهر الأعمدة وجود تباين واضح في سمك الطبقة العمادية بين العينات المختلفة مقارنة مع عينات السيطرة وتاثر سمك العرق الوسطي لورقة *A. marina* المعرضة لنفس التراكيز ، كما تعرضت طبقتي النسيج الكولونكيمي العليا والسفلى في ورقة الى تاثيرات كبيرة اذ ظهر أن سمك النسيج الكولنكيمي في الطبقة السفلى أكبر من الطبقة العليا في جميع العينات، اذ بلغ اعلى سمك لطبقة العليا 106 ميكروميتر عند التركيز 7.5 جزء بالمليون ، بينما بلغ اعلى سمك لطبقة السفلى 162 ميكروميتر عند التركيز الخامس. اما ادنى سمك لطبقة العليا من النسيج الكولنكيمي كانت 35.38 ميكروميتر عند التركيز 2.5 جزء بالمليون اما في الطبقة السفلى فكان ادناها سمك 100 مايكروميتر ايضا لنفس التركيز.

الكلمات المفتاحية: المانكروف، الصفات تشريحية، التلوث، البصرة .