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The Effect of Petroleum Pollutants on the Anatomical Features of *Avicennia marina* (Forssk.) Vierh

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Abstract: ***The present study is the first one conducted to evaluate the effect of oil pollutants on the anatomical structure of one of the most economical plant namely; Avicennia marina, naturally growing at Abu Monqare Island, west coast of the Red Sea, Egypt. In this study, the Leaves and the aerial roots (pneumatophores) are the main studied organs as they are the major parts which were affected by the oil pollution. The pneumatophores are divided into three parts; basal, middle and upper parts, according to its exposure to oil pollution. The obtained results revealed that the extracted residual oil of the contaminated soil contains some harmful substances such as aromatics, saturates asphaltenes and resins. The oil pollutants are deposited inside the tissues of leaves and aerial roots which leads to obvious changes on the anatomical features of the plant. According to this study, it was found that the effect of the petroleum hydrocarbons pollutants may cause serious plant diseases such as chlorosis and tuberculosis and quick decline of the plant in this area.***

Keywords: Petroleum oil pollutants, *Avicennia marina* anatomy

1. Introduction

The term “mangrove” refers to an assemblage of tropical trees and shrubs that grow in the intertidal zone. These zones are frequently inundated with salt water due to tidal activity of gulfs, seas and oceans. The term also refers to the mangrove family of plants, Rhizophoraceae, or more generally to mangrove trees of the genus Rhizophora. The relationship between mangroves and their associated marine life cannot be overemphasized. The mangroves and coral reefs are linked together not only in the ways they protect each other but also by the animals that move between the two habitats. Mangroves play a key role in near shore habitats of the tropical zone (CDA,1996).

All mangrove forests in the Red Sea area, including the Gulf of Aqaba, are pure forests of *Avicennia marina*, occasionally mixed with *Rhizophora mucronata* in the southern part of the Red Sea. Most of these mangrove forests nowadays are protected and managed by the government, at least in Saudi Arabia, Kuwait, Qatar, UAE, Oman, and Egypt. (ken Yoshikawa *et al*. 2011).

Mangroves act as a filtering system for the run-off and ground waters, clarifying adjacent open water, which facilitates photosynthesis in marine plants. It also help to control other forms of pollution, including excess amounts of nitrogen and phosphorous, petroleum products, and halogenated compounds. Mangroves serve as nursery and refuge for many juvenile fish and invertebrates such as spiny lobster, gray snapper, jacks and barracuda. Mangroves are the nesting white-crowned pigeons and frigate birds. Also, it can be used as many kinds of biomaterials, such as building materials, firewood, charcoal, fodder, medicine, etc(Ken Yoshikawa et al., 2011).

Although contamination of PAHs can result from natural and anthropogenic processes, inputs of PAHs from human activities such as oil spill, offshore production, transportation and combustion are very significant and pose serious threats to costal habitats such as mangroves (Ohanmu, E. O. 2014).

Crude oil spills affect plants adversely by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them. It has been recorded that oil contamination causes slow rat of germination in plants. This effect could be due to the oil which acts as a physical barrier preventing or reducing access of the seeds to water and oxygen (Erute, M. O. et al. 2009).

Oil drilling in or near mangrove shorelines has significant adverse impacts (Longley *et al*., 1978). Petroleum oils and their by-products affect mangroves by coating aerial and submerged roots and prevent them from direct absorption (Odum and Johannes 1975, Carlberg 1980). Some severe effects, including tree death, can take place monthly or annually after a spill (Lewis 1979, 1980). Common dispersants used to combat oil spills are toxic to vascular plants (Baker 1971). Damage from the actions of mechanical abrasion, trampling, or compaction during cleanup can exacerbate negative environmental impacts.

Some studies showed that there is an effect of the growth and anatomy of *Amaranthus hybrid*us plant when subjected to petroleum oil pollution, where stomata index of leaves progressively decreased as the crude oil concentration of soil increased. Also the thickness of walls of the cortical cells was more prominent in *A. hybridus* grown in crude oil polluted soil than in the control. The cortical cells of the roots and stems of *A. hybridus* in 2-3% polluted than the grown in control which had round polygonal cells that appeared larger. These changes in anatomy of *A. hybridus* due to contamination by crude oil were discussed as possible pollution indicators.(Omosun *et al*. 2008).

Furthermore, anatomical studies of the treated plants (*Chromolaena odorata*) with crude oil revealed the presence of oil films in the epidermal and cortical regions of the root, stem and leaves. Also the crude oil affects the cell disruption in root and other organs (Gill *et al*., 2005).

However, data on oil polluted mangrove ecosystems in Egypt are not reported or have – as yet- been published. To the best of our- knowledge this study is the first one that evaluate the effect of petroleum oil contamination on the anatomy of *Avicennia marina* plant naturally growing in Abu-Monkar Island, Red Sea, Egypt.

2. Materials and Methods

2.1 Collection of Soil Samples

At Abu-Monkar Island two sites were chosen; site (I) on the north side of the island represents the polluted site, and site (II) on the south side represents the unpolluted one (Fig.1c). It has been observed that the trees grown in the north side are affected by oil pollution throw the current that contain the oil coming from the north and also by the north east wind which strike the trees grown in this side, while those grown in the south were safe from the effect of oil pollution. From each site a sample of soil was collected at a depth of (0-20 cm). The collected soil samples were mixed thoroughly to form a composite sample; they were air-dried and sieved through 2mm sieve to separate the sand from gravel.

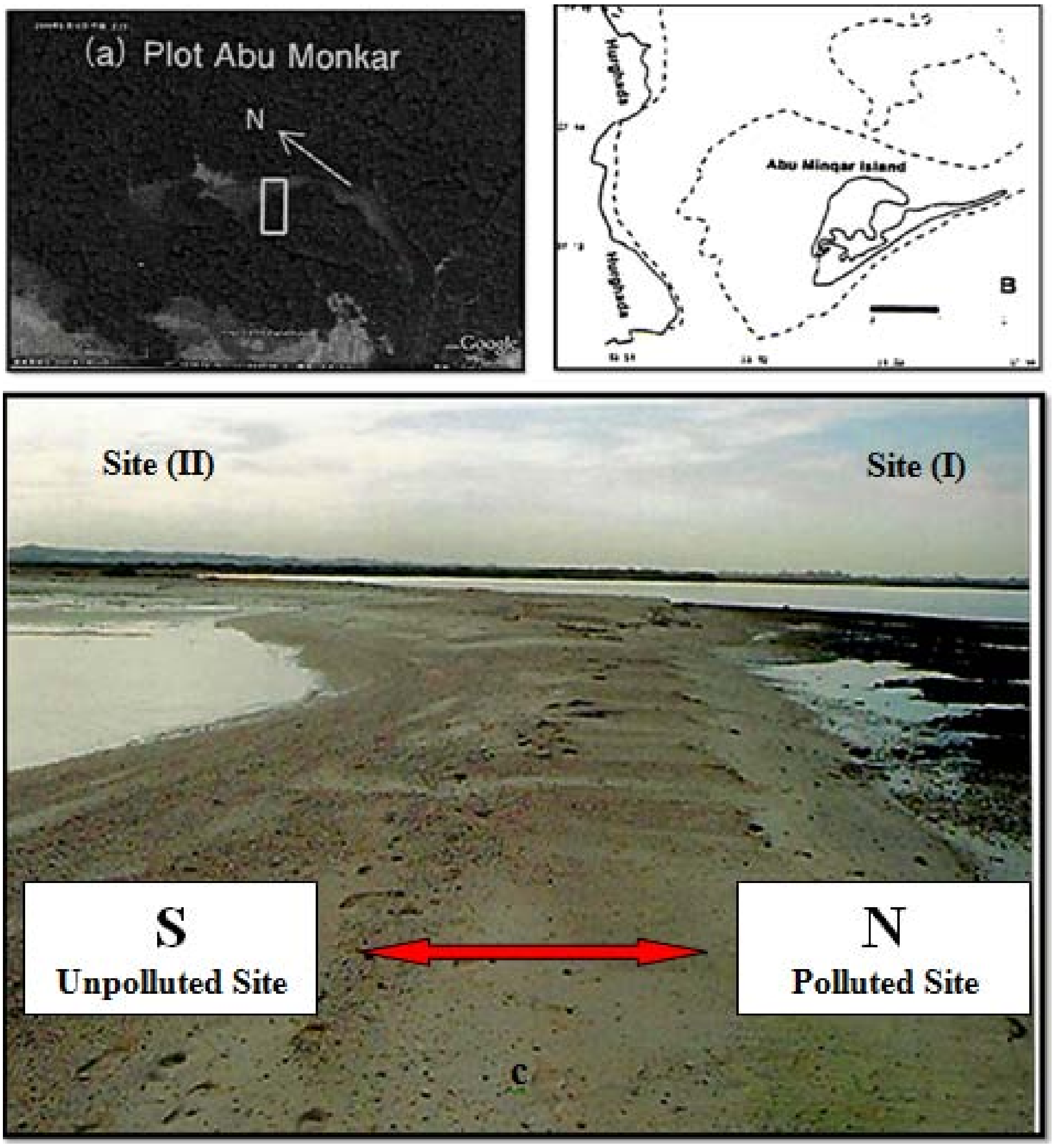


Figure 1: Map of Abu- Monkar shows: (a) Location of the study area (b) Map of the Abu-Monkar Island in details (c) The polluted site (I) [North of the Island] and (II) the unpolluted site [South of the Island]

(1-1) Determination of the Total Oil Content:

The total residual oil of the soil sample was extracted by methylene chloride using the shaking method described by (Chen et al, 1996) as follows:

- Three grams of the sample were introduced into 50ml stopper glass bottle,then 10ml methylene chloride and one gram anhydrous sodium sulfate were added.
- The bottles were shaken for two extraction periods of 60 minutes each using reciprocal shaker operated at 150 rpm, The methylene choloride extract was pooled and evaporated in a preweighed dish.
- The amount of residual oil was determined gravimetrically.

(1-2) Determination of the Different Fractions of the Residual Oil:

A Known weight (100mg) of the extracted residual oil was suspended in n-hexane and filtered through tarred filter paper to remove and to determine the non-soluble fraction asphaltene (Oudot, 1984).

The hexane soluble fraction was fractionated into saturates, aromatics, asphaltenes and resin by liquid chromatography on 100-200 mesh activated silica gel column (15x 1.5cm) by successive elution with 60ml aliquots of n-hexane, benzene and methanol. The solvents were evaporated and each fraction was weighed. The amount of each fraction was then determined as described by Chaineau et al, (1995).

1. Plant sampling:

Mature leaves and different parts of the aerial roots; pneumatophores (top, middle, and basal parts which is embedded in water) of the plant were collected to investigate and compare their micrology at the two sites.

a. Samples were taken from mature leaves on the new growing shoots, specimens were taken halfway from the mid-rip edge, samples also were taken from aerial roots (pneumatophores). Samples from the parts deep near the roots (basal) and also from the upper parts which not immerge on the water (top and middle), all the samples will killed and fixed in F.A.A 70%, after the end of fixation period, specimen where dehydrated in the chloroform method according to Johanson (1940); after dehydration the specimen were embedded in paraffin wax (58%); sections were cut at a thickness 10-12 u by using rotary microtome. The samples were stained with safranin FCF, malachite green, method (Johansan, 1940) then mounted in Canada Balsam. The sections were examined and photographed by Zeiss search microscope.
b. Samples from the leaves and aerial roots (from the previous sites) were dried at (70°C) for seven days. The dried samples (50g) were soxhlet extracted by chloroform and analyzed by the same method as described before.

3. Statistical Analysis

The results were analyzed with computer assistance using the Student's "t-test" using $\alpha = 0.05$ as a level of significance and n=4 as a size for each sample.

4. Results and Discussion

1. Oil content of the soil, plant leaves and aerial roots

Regarding oil content of the soil, data presented in table (1) and Fig.(2) showed that the soil of the polluted area at the north side of the Island contain an amount of total oil contend reached about 3500 mg/100 g dry soil. On fractionation of the extracted residual oil, different harmful compounds were obtained, for example, aromatics, saturates, asphaltenes and resins. These compounds amounted the values of 1050, 1575, 350 and 525 mg/100g dry soil, respectively.

In plant leaves and aerial roots the chloroform extractable materials were 60 mg/Kg and 400mg/Kg dried leaves and dried aerial roots respectively.

Table 1: Amount of the Residual Oil and its Fractions in the Mangrove Polluted Soil

Pollutants	mg/100gm Soil	%
Saturates	1050	30%
Aromatics	1575	45%
Resins	350	10%
Asphaltenes	525	15%
Total Residual Oil	3500	100%

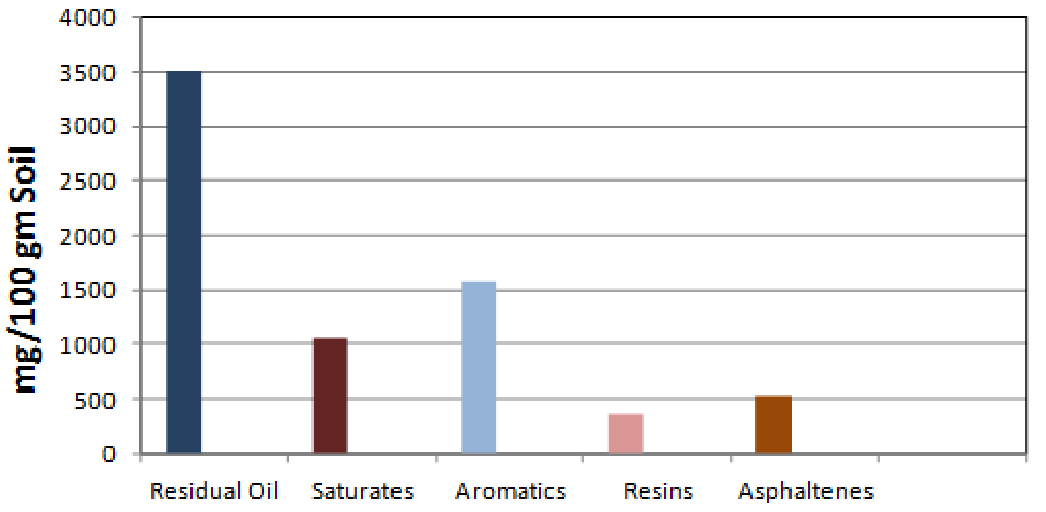


Fig 2 : Amount of the Total Residual Oil and its Fractions in the Mangrove Polluted Soil

2. Anatomical Characteristics:-

The micrology of leaves and aerial roots (Pneumatophors) of the crynohalophytes ***Avicennia marina*** plant was investigated in two sites at Abo-Monqar Island; site (I) lies north side of the island where it exposes to high concentrations of petroleum oil pollutants (3.5%) and represents the polluted site, Fig (1:c) while site (II) lies south side of the island where the sea water is nearly free from oil pollutants and represents the unpolluted site, Fig (1:c). There are distinct variations in growth form of plants at the two sites, symptoms such as chlorosis were observed at the polluted plants as shown in Fig (3a&b).

(a)

(b)

Figure 3: shows the distinct variations in growth form of plants at the two sites: (a) The Polluted Site (I), North of the Island (b) The Unpolluted Site (II), South of the Island

(2-1) Leaf Anatomy:

On the investigation of a cross section of a mature unpolluted leaf as a control sample from site II (Fig 4a&b); it is found that the upper epidermis consists of a single layer of tabular-shaped cells covered with a thin layer of clear hairs known as trichomes, Fig.(4a,T). It is apparent from the cross section that these trichomes contain sulpher-yellow deposits called lapachol. It is believed that trichomes and their constituents of lapachol deposits contrast the extreme sun rays incident on the plant all-over the day. The upper epidermis consists of multi-layered (4-5 layers) spongy or storage cells called hypodermis or hypodermal tissue that represents about 40% of the sector size fig.(4a,E). The innermost layer of the hypodermis has smaller cells than that of the outermost one. Also, all cells of the hypodermis have rather thick walls and have no inter cellular spaces. The surface area of the storage cells (hypodermis) ranged from 171-180 µ. On the other hand, the same tissue in the lower side of the cross section of the leaf has greater cells than that of the upper tissue. This may be due to the upper hypodermal cells continuously exposed to long term of sunshine and lost more water through rapid transpiration process. The upper hypodermis compensates the loss of water from the lower one. Following the hypodermis, the mesophyll tissue that consists of three layers of palisade cells. The outer layer of this tissue has small cells of diameter ranged from $2\times8.4\mu$ to $3.5\times9.5\mu$, while cells of the inner layer have diameters ranged from $6.3\times8.5\mu$ to $4.5\times8.4\mu$. The palisade tissue that contains chloroplasts or green plastids represents about 35% of the leaf sector and responsible for the photosynthesis. The outstanding features in the cross section of the leaf were the lower epidermis covered by a thin layer of cutin of 1-5µ in thickness; fig.(4a,c) and there is a deep seated salt gland on the lower epidermis, Fig.(4b). The observations of the present investigation on the internal structure of the ***Avicennia marina*** plant were in agreement with the findings of Metcalfe and Chalk(1960) and Ahmed *et al* (1999).

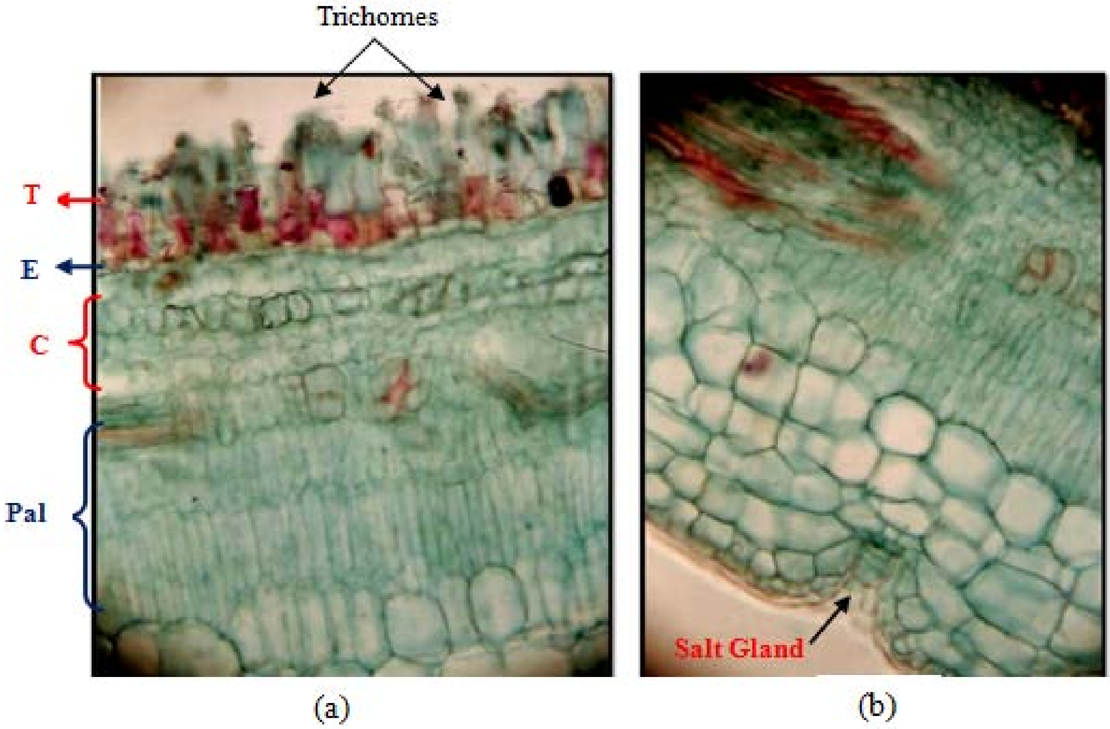
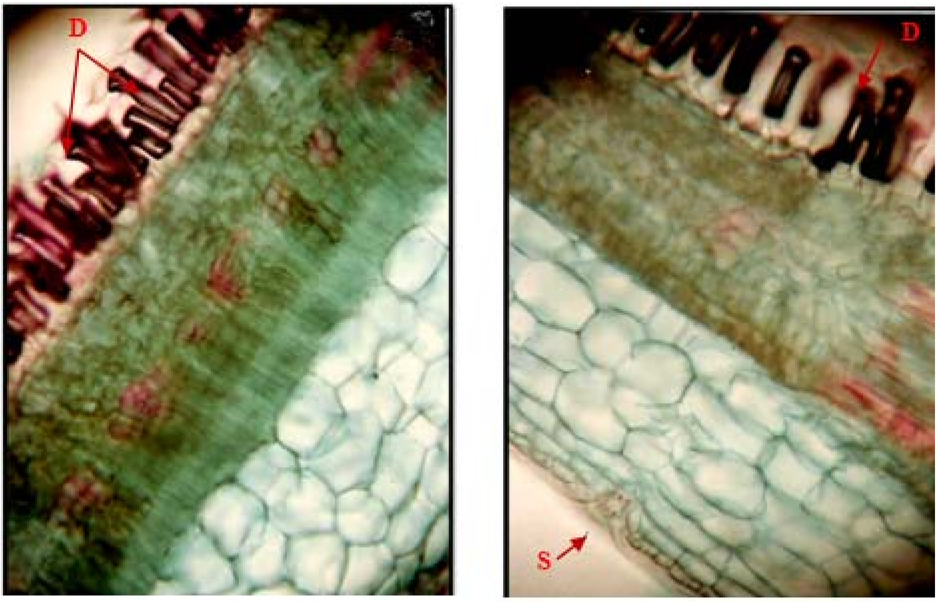


Figure 4: T.S. in mature unpolluted leaf: (a) showing: the upper surface covered with hairs layer trichomes(T) ; epidermis layer (E) ; cutin (C) and palisade cells (Pal). x120 (b) showing the lower surface with the deep seated salt gland. x120

On the other hand, owing to high levels of oil pollutants on the north side of the island, site (I), the internal structure of the leaves of ***Avicennia marina*** plant have much been affected as shown in Fig.(5a&b).On comparison the cross section of mature polluted leaf with that of unpolluted one, we can recognize the following variations :

- Surface of the upper epidermis became covered with thick layer of dark hair or trichomes. This may be due to the deposition of certain materials, either through evaporating oil or through absorption processes by roots. Accordingly, the appearance of the cross section of the leaf seems rusty and dark in color, Fig. (5 a&b).
- The upper epidermal cells became very compacted and smaller in size and consequently its surface area decreased.
- The multiple- layered of the hypodermis, that represents about 40% of the unpolluted (control) cross section became much compacted with smaller cells in the polluted cross section, Fig. (5a).
- The palisade tissue also became much compacted with smaller cells. Hypodermis and palisade tissues (mesophyll) became darkness due to the presence of strange materials inside the tissues as a result of oil pollution. Also, scleride sheathes were observed between cells of the mesophyll tissue.
- The lower epidermal and the lower hypodermal cells became elongated and small in size. A thick cutin layer (about 2µ) covered the lower epidermis. This may leads to exposing the plant to rapid destruction and death.
- The most outstanding feature was that the salt gland became superficially seated on the lower epidermis and became smaller in size, Fig.(5b).

In this regard, results of the present investigation were in agreement with those of Gill *et al.*, (2005) and Omosun *et al.*, (2008) where they demonstrated that oil pollution affects the internal structure of ***Chromolaena odorata*** and ***Amaranthus hybridus*** plants.



(a) (b)

Figure 5: T.S. in mature polluted leaf (a) showing **D** the dark Trichomes. X120 (b) showing **S** the superficially seated salt gland. X120

(2-2) Anatomy of the aerial roots (pneumotophores):

Mangrove plants produce large numbers of stilt roots from the main stems and branches. In many cases, in addition to the stilt roots special roots called respiratory roots or pneumatophores, are produced in large numbers, such roots develop from underground roots. They provided with numerous respiratory pores in the upper part, through which exchange of gases takes place. It was observed that the black spots of petroleum oil on the pneumatophores prevent the exchange of gases leading to damping off the plant and consequently the plants died, Fig(6).

It had been found that these important organs were much affected, either externally or internally, by oil pollutants where they are directly inundated by water. In the present study, the individual pneumatophore was divided into three parts; upper(over the sea level), middle(at the sea level) and the most affected basal part(under the sea level).

(a) (b)

Figure 6: showing the aerial roots pneumatophores: (a) anvil- like shapes (b) the polluted pneumatophores; showing black spots of petroleum oil.

(2-2-1) Anatomy of the basal part:

It is clear, owing to petroleum oil pollution, that the great damage was found in the basal zone of the aerial root (pneumatophore) where it always being sunks in water as shown in Fig(6). The polluted zone exhibited different internal changes if compared with the healthy one, Fig. (7 a & b). These variations can be summarized as follows:

- The diameter of polluted cross section of the basal part measured about 9.1mm, while it measured 6.1, in the same zone of the healthy root. The increasing in diameter of polluted basal zone of pneumatophore may be due to the increment of the phellogen tissue (cork cambium arising as secondary meristem giving rise to cork and phelloderm) that sometimes reach up to 12 layers filled with dark deposits, Fig. (7b). In cross section of the healthy (unpolluted) zone of the aerial root, the phellogen tissue not exceed 5 layers as shown in Fig. (7a). Also, crushed epidermal cells outlined with cuticle were clearly appeared in the polluted basal zone of the pneumatophores.
- The ground cells of the polluted basal zone of the root had thicker cell walls, Fig. (7b), than that of the same zone of the unpolluted (healthy) root, Fig. (7a) which have clear intercellular spaces.
- The very fine ingredients of the oil pollutants attack the cork tissue (a tissue derived from outer layer of cortex) and destroy it. At the same time, some tar deposits in the ground tissue of this zone of the root also attack the newly developing secondary root (rootlet), as shown in Fig. (8).
- The oil-soluble materials encouraged the formation and increasing of malignant cells that divide irregularly and rapidly (destructive or destroyer cells) that appear as a mass of small cells containing large nuclei, Fig. (9a). ultimately, these cells (malignant) gradually develop giving rise or providing a tumor as shown in Fig. (9b).

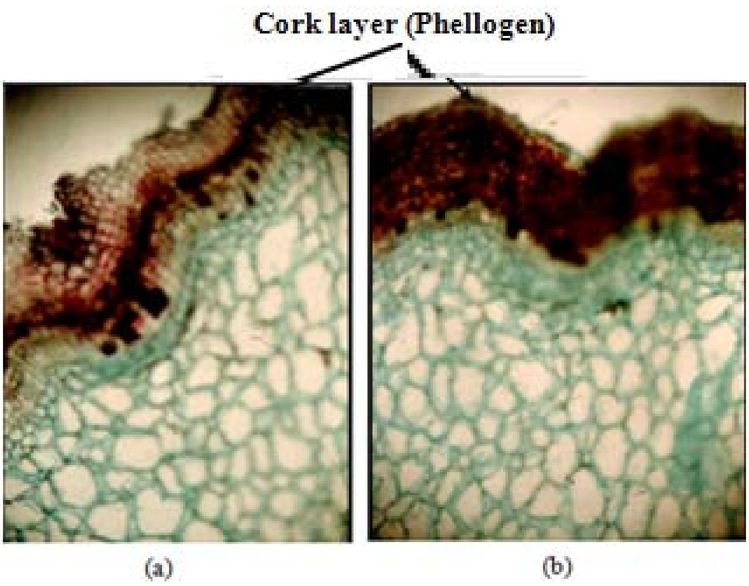


Figure 7: T.S. in basal zone of pneumatophores: (a) unpolluted. x180 (b) polluted. x180

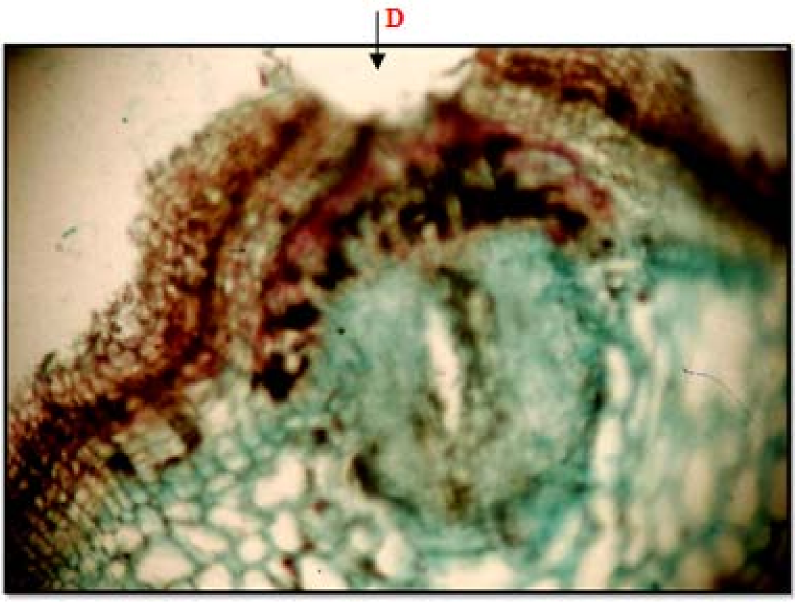
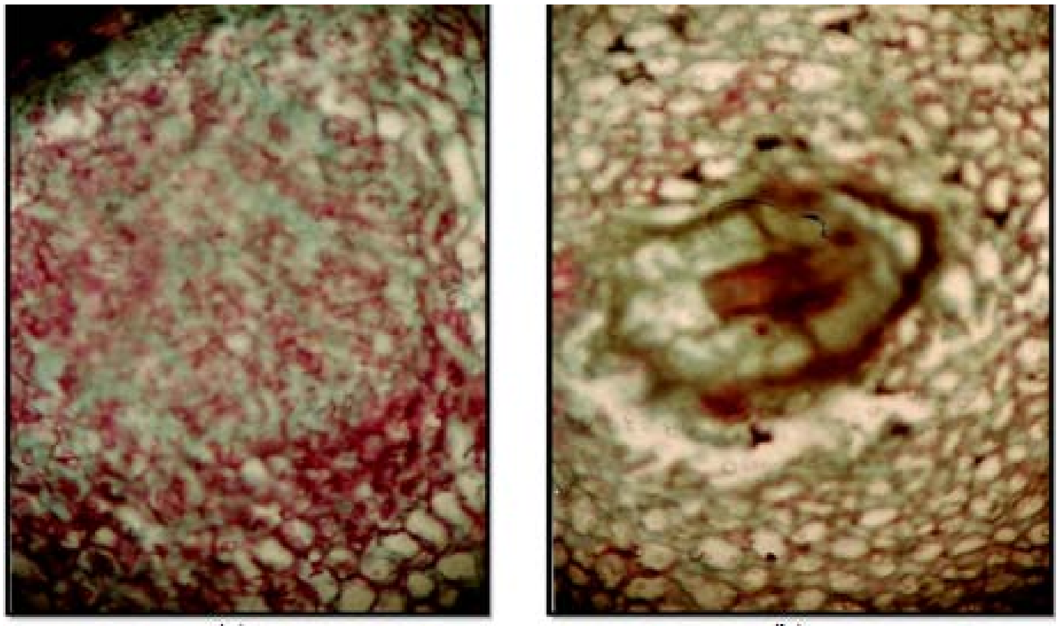


Figure 8: T.S. in basal zone of pneumatophores showing D (the destroyed cork tissue) due to fine ingredience of oil.x180

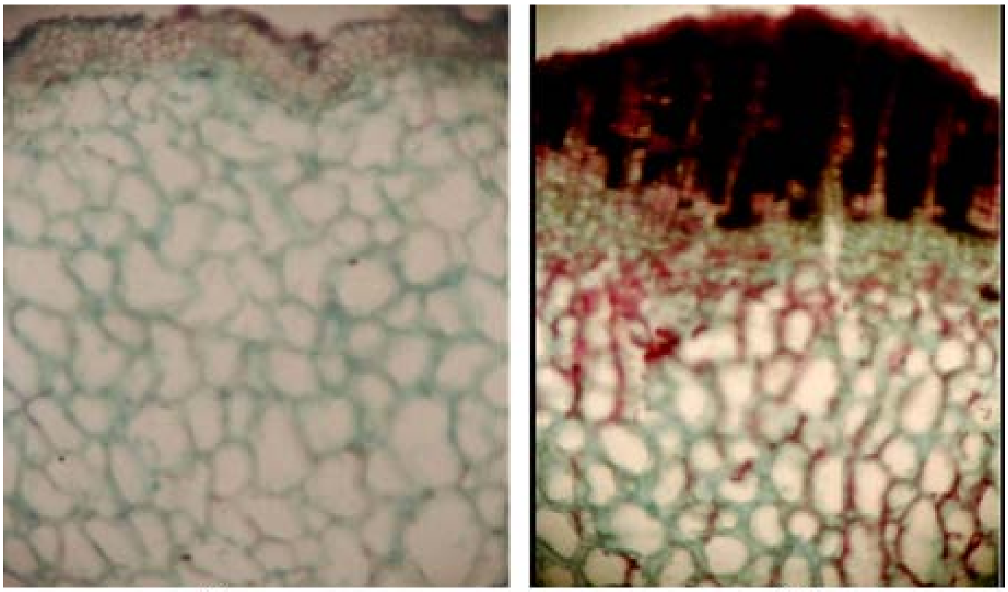


(a) (b)

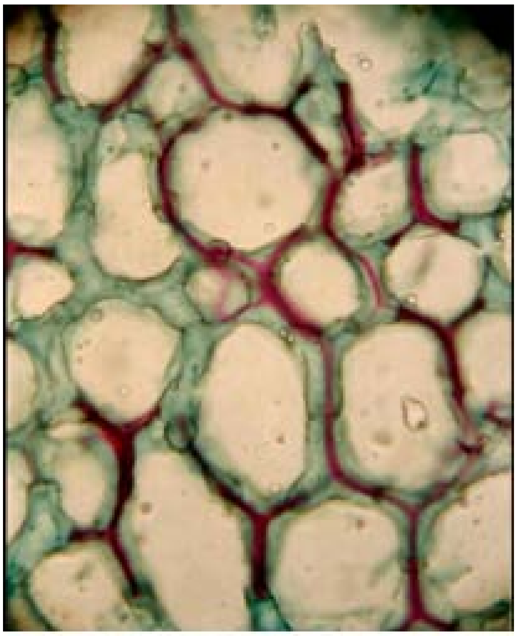
Figure 9: T.S. in basal zone of pneumatophores showing: (a) malignant cells. x180 (b) Tumor. x180

(2-2-2) Anatomy of the middle part:

This part usually is exposed to tide waves fluctuation; i.e oil pollutants still affect it. The diameter of the cross section of this part reached about 7.6mm. The cork layer still dark filled with dark deposits of petroleum oil, cork tissue had been more thickness in the contaminated plant that help to protect the inner tissues from the toxicity of the pollutants, Fig. (10b). At the same time, it was found the appearance of insoluble substances between the ground cells filling the intercellular spaces of all the ground tissue, not inside the cells to protect the living cells from damage. Fig.(10c). On comparison, Fig. (10a) shows the healthy (unpolluted) middle cross section of the root where the cortex tissue seems light dark with limited uncutinized cells. The ground tissue had cells of thin walls with clear intercellular spaces. The epidermal layer appeared as entire surface with very little ruptures (lenticels). In the light of the above observations for polluted basal and middle parts, the injuries by petroleum oil pollutants cause a great reduction in the efficiency of the ground tissue of these parts.



(a) (b)

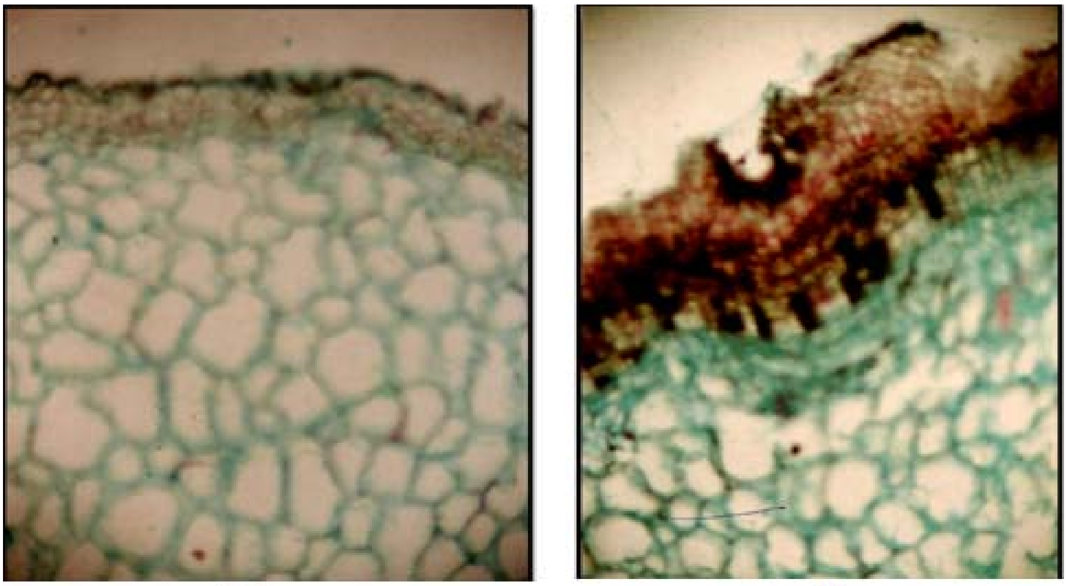


(c)

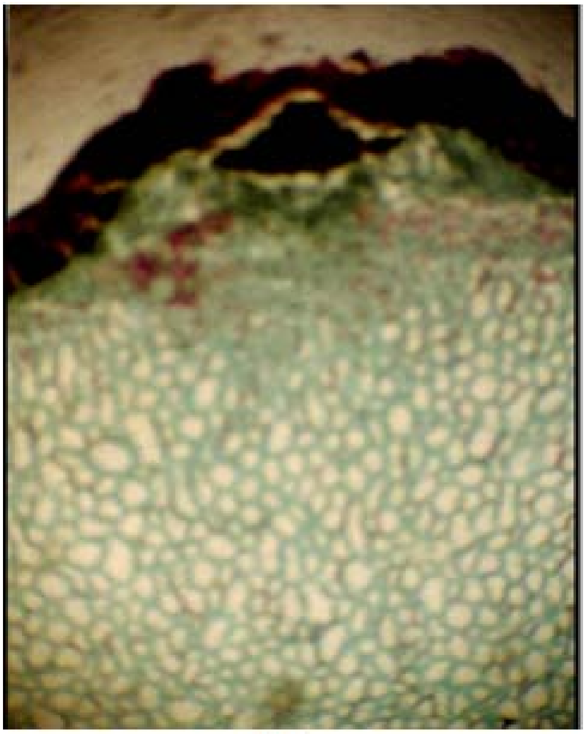
Figure 10: T.S. in middle zone of pneumatophores showing : (a) unpolluted cross section x120. (b) polluted cross section x180. (c) close-up view of cortex tissue with intercellular spaces filled with insoluble substances. x180

(2-2-3) Anatomy of the upper part:

Although the upper parts of the aerial roots projecting beyond the water and they look like so many conical spikes, sometimes ending with anvil-like shape Fig. (6a), but they still slightly affected by oil pollutants in site (I). The cross section of the polluted upper zone seems thinner in its diameter, recording 4.5mm, than that of unpolluted one which recorded 5.2mm in its diameter. On comparison, the unpolluted cross section exhibited the following features, Fig. (11a) :



(a) (b)



(c)

Figure 11: T.S. in upper zone of pneumatophores showing: (a) unpolluted cross section. x120 (b & c) polluted cross section showing the rupture tissues. x120, x180

- The outer layer (epidermis) was entirely raptured or interrupted by several lenticels (ventilating pores) that permit the air to inters and reaches the inner layers. So, the upper part of the pneumatophores is considered as an essential part because it allows respiration process to takes place through numerous lenticels (ventilating pores) spreading on the epidermal layer.
- The phelloderm (parenchymatous suberous cortex) were clear and the ground cells had thin walls with obvious intercellular spaces.

On the other hand, in the polluted cross section, it was found that the cortex became thick with numerous layers filled with tannins and tar. Also, insoluble dark materials deposited in the air spaces of the cortex tissue, Fig. (11b). The ground tissue had cells of thick walls with irregular intercellular spaces.Distinct air bladders of different sizes were also found in the same tissu as shown in Fig. (11c).

It is clear from the above results that the development of abnormality in the different root parts and leaves of the mangrove *Avecinnia marina* is a result of oil pollution of the atmosphere in which this plant grow. The oil pollutants may accumulate in the different tissues and affect the anatomical feature of the mangrove plant.

The accumulation of the hydrocarbon in the different parts of roots, although it affects the anatomy of these parts, it gives an indication for the ability of this mangrove plant to remove oil from its polluted habitat, this leading to the suggestion that mangrove may be useful for phytoremediation strategy.

Youwei, et al (2009) showed that the accumulation of the aromatic hydrocarbons was increased in the roots of mangrove Kandelia Candel by increasing such compounds in the soil. This indicate the ability of the mangrove to remove the aromatic hydrocarbons from the contaminated marine habitats, and hence their potential use in bioremediation and the removal of the pollutants from the marine and food chain. The same authors suggested that direct contact with polluted soil could be the primary roul's for hydrocarbons transporting into roots of mangrove.

5. Conclusion

From anatomical point of view, modified plant organs or tissues resulting from sever environmental stresses are functions of these adverse conditions. So, anatomical studies of desert plants distinctly reveal to what extent that the vital physiological processes are affected by different environmental stresses; drought, salinity and /or pollution. *Avicennia marina* trees are belonging to the mangrove ecosystems that grow besides oil polluted soil in some cases, in particular combined adverse conditions of warm, humid, salty and water-logged environment. This plant acquired high levels of selective adaptability mechanisms both in function and structure to the previous condition by differed degrees according to the type of stress. From the results of the present study we can conclude that :

Anatomically, results of the present investigation evidently shows that the cross sections of healthy (unpolluted) leaf and aerial or respiratory root (pneumotophore) exhibit some peculiarities in their internal structure where leaves are dorsiventral exhibiting a distinct layer of thick cutin covered the upper and lower epidermis. A single – layered epidermis of small cells with multi cellular salt gland on the lower epidermis. The upper epidermis is covered by dense hairs of anvil – like heads (trichomes) that filled with sulfur – yellow substances called lapachol. It is believed that these trichomes and their constituents of lapachol act as a reflective agent for the extreme sun rays incident on the leaves all over the day. The hypodermis of the lower surface has greater cells to compensate the loss of water through rapid transpiration that takes place from the upper surface of the leaves due to long-term exposure to sunshine. The mesophyll consists of multi-layers of palisade and spongy (storage) tissues of clear inter cellular spaces.

Pneumatophores (respiratory roots) play an important role for prosperity and survival of ***Avicnnia marina*** plant in the particular combined conditions of warm, humid, salty and water-logged environment, where the cross section of the healthy root exhibits cork layer that interrupted by numerous lenticels at several spaces along the epidermis, through which (ventilating pores) respiration takes place. The cortex and the ground tissues are extensively aerenchymatous constituting the main bulk of the cross section of the root.

Owing to the petroleum oil pollution found on the north side of Abu-Minqar Island and its deteriorative effects on ***Avicennia marina*** plant, the peculiariries of the internal structure of leaf and pneumatophores exhihibited several negative changes such as :

Firstly in leaf

- The layer of hairs or trichomes became dark and rusty in its appearance due to the deposition of fine ingredients of oil pollutants on the upper surface of the leaves.
- Compactness, darkness and decrement of the surface area of the epidermis, the hypodermis and the mesophyll tissue (palisade and storage cells). Moreover, the lower epidermis seemed covered by a thick layer of cutin.
- Appearance of sclaride sheathes (cells with lignified walls) between cells of the mesophyll tissue.
- The salt glands on the lower epidermis of the leaf became superficially seated and smaller in size with much more compacted cells.

Secondly in Pneumatophores

- Increasing in the diameter of polluted pneumatophore may be due to the increment of phellogen tissue (cork cambium arising as secondary meristem activity giving rise to cork and phelloderm) as a result of pollution stress.
- Very fin ingredients of oil pollutants attacked, crushed and destroyed the epidermis and the cork tissues. Also, some tar and tannins were deposited in the ground tissue and also attacked the newly developing secondary roots (rootlets).
- The most outstanding feature was the appearance of malignant cells (destructive or destroyer cell) as a mass of small cells that finally developed into a tumor causing plant death.
- Appearance of dark insoluble substances between some ground cells and sometimes filling the intercellular spaces of all the ground tissue.
- Ultimately in the light of all the observations for the polluted plants, the present investigation concluded that the all injuries resulting from petroleum oil pollutants cause a great reduction in the vital physiological processes and in the efficiency of all plant tissues leading to the destruction and hidden of the plants. Pathogenically, from the foregoing results, petroleum oil pollution causes distinct and serious diseases to *Avicennia marina* trees such as chlorosis and tuber culosis or parenchymatis. The more pollution by hydrocarbons on this plant in this area may lead to disappearance of vegetation.

6. Recommendation

On long run, because of the petroleum oil contamination and the anthropogenic activities in marine environments are considered as a weapons of massive destruction for mangrove and other marine ecosystems all over the world, so further studies must be done to find out scientific methodology for bioremediation of mangrove ecosystems as a landscaping and as an important nursery habitat for marine and terrestrial living organisms (animals, fish and birds).

The present study suggests using simple, save and cost effective strategy such as specific strains of bacteria capable to rapid get rid of oil pollutions through bio surfactant theory. We must give recommendations to oil tankers to avoid throwing any petroleum oil before entering Swize Canal

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