Ecophysiological Responses of Grey Mangrove (*Avicennia marina*) (Forssk.) Vierh. to Oil Pollution at Ras Mohammed Protective Area

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Abstract: The current study was undertaken to investigate the adverse effect of oil spills on the performance of *Avicennia marina* grow in Ras Mohammed protected Area. The plant materials were collected from four different plots of studied area. The effects of oil pollution on the sediment analysis, photosynthetic pigments, lipid peroxidation as malondialdehyde accumulation, enzyme activities, amino acids, total phenols, total soluble proteins, carbohydrates, and organic acids, of *Avicennia marina* grow in Ras Mohammed protected Area were analyzed. The data cleared significant decrease in the content chlorophyll a, chlorophyll b, and carotenoids, in *Avicennia marina*. The Most significant decrease was observed in plot1 and the highest one in plants growing in plot 4. The significant increasing malondialdehyde (MDA) contents in *Avicennia marina* growing in plot (1) in compassion to *Avicennia marina* growing in plot (4) sites. The activity of peroxidase was significantly increased under oil pollution in *Avicennia marina*. The highest value was detected in plot (1) and lowest value was cleared in plot (4) plants. On the other hand, the activity of catalase, superoxide dismutase and glutathione reductase significantly decreased in *Avicennia marina* growing in plots 1. The highest activity of these enzymes detected in plants growing in plots 4 of the studied area of Ras Mohammed. *Avicennia marina* growing in plots (3,4) contained higher concentrations of aspartic acid, serine, glutamic acid, glycine, alanine, valine, leucine, lysine, threonine, isoleucine, argnine and ammonia than plots (1,2), while Phenylalanine detected only in plant growing in plots (3,4). Proline content was increased in *Avicennia marina* plants growing in plots (1, 2) as compared to *Avicennia marina* plants growing in plots (3, 4). The most significant increases in total phenols, total soluble protein and carbohydrate in *Avicennia marina* plants growing in plot1 and the significant decreases was observed in plants growing in plots 4. The total carbohydrate contents show a reverse pattern. Oil pollution caused significant increase in the contents of citric acid, jasmonic acid and salicylic acid. The highest value was determined in *Avicennia marina* plants growing in plot 1 and the lowest value was cleared in plants growing in plot (4). These changes were discussed in relation to plant performance under environmental pollution.

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Key words: *Avicennia marina*, oil spills, Ras Mohammed protected area.

Introduction:

Mangrove habitats and individual tress are highly sensitive to changes in coastal water quality, especially from the impacts of large petroleum spills **(Duke *et al*., 2000).** Mangroves in Egypt are distributed over numerous small stands from the northern latitudinal limits of the Indo-Pacific East African mangroves realm- The mangroves are species-poor. Thus, only two species of mangroves are recorded in Egypt with *Avicennia marina* the most widespread. Around the world, people utilize mangrove ecosystems particularly for food and forest products.

Avicennia marina in Ras Mohammed protected area is facing a pig problem of oil spills whit have severe, adverse effects on fragile and sensitive natural resources. Preventing the discharge of oil, or a threat of such a discharge, is the most logical means of avoiding problems associated with oil Vegetation studies were carried out on four previously assessed monitoring plots at Ras Mohammed, Sinai, Egypt **(Benfield, 2002)**.

The current study was, therefore, undertaken to analyze the effect of oil spills on sediments of different plots, photosynthetic pigments, carbohydrates, organic acids, lipid peroxidation and antioxidant enzyme activities of the studies plant in the different plots.

2. Material and Methods

1-Studies of plant

Avicennia marina (Forssk.) Vierh. (Family: Avicenniaceae)

Tree or shrub, with elliptical, entire, opposite leathery leaves tapering into a short petiole. Leaves green on the upper surface, snowy white on the lower. Inflorescence dense capitate, terminal on the branches or in their upper leaf axils, 2 or 3 together. Flowers sessile each with a bract and 2 ovate bracteoles, which are silky on the outer surface and shorter than the calyx. Stamens hardly extracted (Montasir and Hasssib, 1956 and Batanouny, 1981).

Plate (1): ***Avicennia marina***

Plate (2): ***Avicennia marina***

2- Study area

Ras Mohammed protected area is located at the meeting point of the Gulf of Suez and Aqaba Gulf.

3-Ecological studies

Sinai Peninsula Vegetation studies were carried out on four previously assessed monitoring plots (Benfield, 2002). Each plot measured 10 m × 10 m and methods were followed as described by English *et al.* (1997) and Benfield (2002).

Three replicate sediment samples from the upper 5 cm of sediment over an area of approximately 5 cm × 5 cm were randomly collected from each plot using a clean, acid-washed plastic scoop. Samples were stored in clean acid-washed plastic containers until transportation to the laboratory. Individual sediment samples were wet sieved through a 1 mm bronze mesh with distilled deionized water and collected in a clean, labeled, acid-washed glass jar. The samples were left in a closed running fume cupboard for approximately one week. Samples were then oven dried at 60˚C ± 5 ˚C for 24 hrs to eliminate any remaining water content. Samples were homogenized and stored in a dry acid-washed plastic bag for metal analysis.

Leaves were collected from each of the four plots. Leaves were collected from trees that were greater than 1m tall with a girth at breast height of greater than 2.5 cm and that were of similar health condition (as determined by degree of predation on leaves).

Determination of total petroleum hydrocarbon (TPH):

This was determined in accordance with American Society for test and materials-ASTM D3921 and D5369.

Chemical analyses of plant materials

1. Photosynthetic Pigments:

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were colorimetrically determined according to Metzner *et al.* (1965).

2- Lipid peroxidation:

The level of lipid peroxidation was measured by determining the levels of malondialdehyde (MDA) content using the method of Hodges *et al.* (1999).

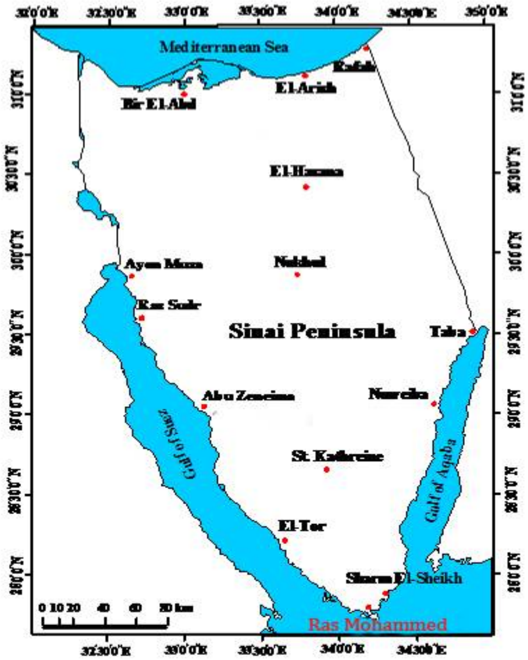

Fig. (1): The Map of Sinai showing the study area

Enzyme extraction:

Fresh Leaf samples were prepared according to MuKherjee and Choudhuri (1983), described sample preparation.

a) - Peroxidase (POX) assay:

POX activity was assayed using a solution containing 5.8 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the enzyme extract and 2.0 ml of 20 mM H_2O_2 (Bergmeyer, 1974).

b) - Catalase (CAT) assay:

CAT activity was assayed according to the Method of Kong *et al.* (1999) and Chen *et al.* (2000).

c) - Super oxide dismutase (SOD) assay:

SOD activity was measured according to the method of Dhindsa *et al.* (1981).

d) - Glutathione Reductase Assay

Glutathione reductase activity was assayed according to Schaedle and Bassham (1977).

6- Estimation of amino acids:

Amino acids compositions of studied leaves protein were hydrolysis and determined according to Catalog of Amino Acid Analyzer (1999) .

Extraction and estimation of total phenols

Total phenols were estimated following the method of Malik and Singh (1980).

Determination of total soluble proteins

The total soluble protein concentration was extracted and determined spectrophotometrically using the Bio-Rad protein assay which is a dye-binding assay (Bio-Rad Technical Bulletin 1051, 1977) and (Bradford, 1976).

Estimation of total carbohydrate contents:

Total carbohydrate content was determined colorimetrically according to Dubois *et al.* (1956).

Estimation of some organic acids

Estimation of citric acid

The method of determination of citric acid was described by Snell and Snell (1949).

Estimation of Jasmonic and Salicylic by High Pressure Liquid Chromatography:

The method used in jasmonic, salicylic extract was described by Matsuura *et al.* (2009).

3. Results and Discussion

Mechanisms of Oil Toxicity to Mangroves

It is clear from spills, and field and laboratory studies, that at least in many Circumstances oil harms or kills mangroves. What is less obvious is how that harm occurs and the mechanism of toxicity. Although there is some consensus that oil causes physical suffocation and toxicological/physiological impacts, researchers disagree as to the relative contributions of each mechanism, which may vary with type of oil and time since the spill (Proffitt *et al.,* 1995).

One of the universal challenges faced by resource managers and spill responders when dealing with oil impacts is the fact that “oil” is a complex mixture of many kinds of chemicals. The oil spilled in one incident is almost certainly different from that spilled in another. In addition, oils within broad categories like “crude oil” or “diesel” can be vastly different, depending on the geological source of the original material, refining processes, and additives incorporated for transportation in barges or tankers. Even if we could somehow stipulate that all spilled oil was to be of a single fixed chemical formulation, petroleum products released into the environment are subjected to differential processes of weathering that immediately begin altering its original physical and chemical characteristics. As a result, samples of oil from exactly the same source can be very different in composition after being subjected to a differing mix of environmental influences.

Soil factors play important roles in plant growth, chemicals composition and distribution of plant under different habitat condition. In the present study sediment soil samples, were collected from 4 different plots in Ras Mohammed protected area.

1-Sediment samples and hydrocarbons analyses:

Results of chemical analyses on sediment samples from the four plots are shown in table (1) all samples had significant amounts of biogenic hydrocarbons. These were distinguished by large odd carbon numbered n-alkane peaks in chromatograms of the saturates fractions. All samples had considerable amount of organic matter (root matting and small fibrous roots) in them and this may be the source of biogenic in them.

All samples showed Ultraviolet (UV) fluorescence which indicated the presence of traces of petroleum. The samples with lowest Ultra violet UV max were from plot (4) and plot (3). This was in agreement with the low aromatic hydrocarbon content of these samples.

Table (1): Results of chemical analyses of sediment samples from different plots (mean±SD)

Sample	Saturates μgg^{-1} dry.wt	Aromatic μgg^{-1} dry.wt	Total Hydrocarbons μgg^{-1} dry.wt
Plot 1	86± 6	32 ± 6	118 ± 10
Plot 2	65±9	15 ± 4	80 ± 13
Plot 3	20± 2	5 ± 2	25 ± 2
Plot 4	10 ± 1	2 ± 1	12 ± 2

Levels of parent Polycyclic Aromatic Hydrocarbon (PAH) compounds are shown in table (2) while there is deference between samples from plots (1) and (3) the concentrations were not particularly high. The parent Polycyclic Aromatic Hydrocarbons PAHs were indicative of combustion sources, as their concentration in oil was not particularly high. The alkylated Polycyclic Aromatic Hydrocarbons PAHs were present in several samples from plots (1) & (2) confirming the presence of chronic oil contamination in these samples. In consideration of the alkylated Polycyclic Aromatic Hydrocarbon PAH (C1 – C3 naphthalenes and C1 – C3 phenanthrenes 1 anthracenes) fractions, the results showed that the naphthalenes were in all cases below the detection limit. However some alkyl phenanthrenes lanthracenes were found .at low concentrations in most of the samples. The results suggest some petroleum contamination of the samples. From plot (2) were unusual in that concentrations were expected to increase from C1 to C3 compounds. However in these samples the concentrations decreased from C1 to C3. This may be due to some co-eluting compounds or some other unspecified interference. The pattern of alky Polycyclic Aromatic Hydrocarbon PAH concentrations for samples from plot (1) were more of what is expected in typical oil samples although, the concentrations are relatively low. This is the pattern expected for sediment subjected to chronic oil. All samples showed Ultraviolet UV fluorescence which indicated the presence of trace of petroleum, but the samples with the lowest Ultraviolet UV max were from plot (4). This was in agreement with the low aromatic hydrocarbon content of these samples. Sediment samples from the four plots had significant amounts of biogenic hydrocarbons. Levels of parent Polycyclic Aromatic Hydrocarbon (PAH) compounds showed deference between samples from plots (1) and (3) the concentrations were not particularly high. The alkylated Polycyclic Aromatic Hydrocarbons PAHs were present in several samples from plots (1) & (2) confirming the presence of chronic oil contamination in these samples. Similar observations were reported earlier by Getter *et al.* (1985). These observations are compelling support for the proposal that petroleum hydrocarbons are responsible for causing, or favoring, the occurrence of lethal mutations in mangrove plants. However, for A. marina, the levels of total hydrocarbons in sediments in plot (3) were lower than in plots 1&2. It is of great concern, for instance, that valuable mangrove habitat may be threatened further by a loss of genetic fitness in the presence of petroleum hydrocarbons in sediments. Petroleum hydrocarbons are known to persist in mangrove sediments for several decades (Burns *et al.,* 1993), and trees take a similar time to mature (Duke, 2001), so there are several factors which might influence mutant gene frequencies and cause mutations.

The magnitude of the oil effect was greater in some plots than in others and plants in general grew better in some plots. An important factor not taken into account in the experiment was the size of sediment particles and the degree of water logging of the sediment. Each of the areas had a range of sediment types from fine salty mud to sand so it was inevitable that sediment sizes in plots would vary. It was noted that those plots experiencing the greatest mortality were plots with fine sediments that never seemed to drain. Dicks (1986) observed a similar interaction between sediment size and the effects of oil though this was not experimentally tested. Little (1987) also found that sediment grain size and moisture content influenced the residence time of crude oil in the sediment and that mud particles tended to retain surface-bound contaminants more strongly than sands. In the longer term, the plot effect proved not be significant in our research.

Underwood & Peterson (1988) suggested that the first step after rejecting a null hypothesis that there is no effect of a pollutant is to determine whether the observed effect really matters. The results of the field oiling experiment on seedlings are unequivocal but are this biologically significant? Clarke & Allaway (1993) have shown that seedlings are unlikely to recruit to the sapling and adult populations unless they establish in large canopy gaps with sediment disturbance or newly deposited sediments. If these places are oiled then recruitment to the adult population will be impaired until a new cohort is able to colonize the regeneration niche. The results of the establishment experiment show that about four times as many seedlings survived on un oiled substrates than on experimentally oiled substrates. A single oil spill may cause widespread seedling mortality and oil in the sediment may inhibit the establishment of propagates but if nearby adults continue to be fecund the long-term consequences may not be serious. It is evident from the literature, however, that mangroves contaminated with oil are often subjected to further oil spills (Dicks, 1986; Snowden & Ekweozor, 1987; Jackson *et al.,* 1988; Corredor *et al.,* 1990). This constitutes a repeated pulse disturbance that may lead to longer term press effects (Underwood, 1989). In particular, the interactions of differing components of the population to repeated disturbances are poorly understood. It is logistically difficult to conduct experiments in which the full range of frequencies, magnitudes, oil types and age classes can be manipulated to assess the impact of perturbations on natural populations. Whilst further experiments are essential for accurately predicting impact, a working hypothesis can be developed based on our results, field correlations, and population experiments. The latter includes reproductive biology.

Table (2): Polycyclic Aromatic Hydrocarbons (PAHs) in mangrove sediments (ngg^{-1} dry wt. of sediment) for four plots (values are mean estimates from four surface samples each.

No of ring	PAH compounds	Plot 1	Plot 2	Plot 3	Plot 4
2	Parent Naphthalene	<5	<5	<5	<5
3	Acenaphthylene	<5	<5	<5	<5
3	Acenaphthene	<5	<5	<5	<5
3	Fluorene	12	19	<5	<5
3	phenanthrene	20	15	<5	<5
3	Anthracene	83	54	55	<5
4	Fluoranthene	30	52	45	<5
4	Pyrene	32	46	20	<5
4	Benz[a] anthracene	32	33	20	<5
4	Chrysene	16	22	20	<5
5	Benzo[b] fluranthene	44	40	60	<5
5	Benzo[k] fluranthene	20	20	15	<5
5	Benzo(a) pyrene	36	40	20	<5
5	Dibenzo(a-h) anthrathene	78	55	<5	<5
6	Indeno[1,2,3-cd] pyrene	20	<5	<5	<5
6	Benzo[g,h,i] perylene	43	35	10	<5
	Total	466	431	265	-
	Alkylated				
	C1 Naphthalene	<5	<5	<5	<5
	C2 Naphthalene	<5	<5	<5	<5
	C3 Naphthalene	<5	<5	<5	<5
	C1 phenanthrene 1 anthracene	44	53	<5	<5
	C2 phenanthrene 1 anthracene	50	30	<5	<5
	C3 phenanthrene 1 anthracene	66	15	<5	<5

2-Plant analyses

2.1. Leaves samples and hydrocarbons analyses

The hydrocarbon concentration in the leaves samples were all quite low compared with those results of sediment analyses (20- 2.2 µgg-1 dry weight).

Table (3) Results of chmical analyses of leaves of different plots (Mean±SD).

Sample	Saturates µgg^{-1} dry.wt	Aromatic µgg^{-1} dry.wt	Total hydrocarbons µgg^{-1} dry.wt
Plot 1	15± 0.2	5± 0.1	20 ± 0.2
Plot 2	10±0.2	3 ± 0.3	13 ± 0.3
Plot 3	3.3± 0.05	2± 0.1	5.2 ± 0.2
Plot 4	1.2 ± 0.03	1 ± 0.1	2.2 ± 0.1

Levels of Parent Polycyclic Aromatic Hydrocarbon PAH compounds are shown in Table (4) Naphthalene, Acenaphthylene and Acenaphtene were not detected in the four plots. Tri-aromatic represented the highest value in both plot (1) and (2). Tetra, penta and hexa aromatic hydrocarbons also followed no particular order. On the other hand plot (4) all hydrocarbons was not detected. The majority of studies show few significant correlations between metal levels in sediment and metals in tissues, which suggests that mangroves actively avoid metal uptake and/or most metals are present below the sediment bioavailability threshold (MacFarlane *et al.,* 2003). Mangroves have developed a complex series of physiological mechanisms to enable them to survive in a low-oxygen, high-salinity world. A major point to remember in terms of physical effects of oil spills on mangroves is that many, if not most, of these adaptations depend on unimpeded exchange with either water or air.

Table (4): Polycyclic Aromatic Hydrocarbons PHAs in mangrove leaves

No. of rings	PAH compounds	Plot 1	Plot 2	Plot 3	Plot 4
2	Parent Naphthalene	ND	ND	ND	ND
3	Acenaphthylene	ND	ND	ND	ND
3	Acenaphthene	ND	ND	ND	ND
3	fluorene	11	20	ND	ND
3	phenanthrene	12	13	ND	ND
3	Anthracene	10	12	ND	ND
4	Fluoranthene	ND	ND	ND	ND
4	Pyrene	15	12	ND	ND
4	Benz[a] anthracene	11	15	13	ND
4	Chrysene	17	13	12	ND
5	Benzo[b] fluranthene	13	18	15	ND
5	Benzo[k] fluranthene	12	12	13	ND
5	Benzo(a) pyrene	33	23	<5	ND
5	Dibenzo(a-h) anthrathene	17	21	<5	ND
6	Indeno[1,2,3-cd] pyrene	12	<5	<5	ND
6	Benzo[g,h,i] perylene	22	15	<5	ND
	TotalPAHs	185	174	64	0
	Alkylated				
	C1 Naphthalene	ND	ND	ND	ND
	C2 Naphthalene	ND	ND	ND	ND
	C3 Naphthalene	ND	ND	ND	ND
	C1 phenanthrene 1 anthracene	22	18	ND	ND
	C2 phenanthrene 1anthracene	28	10	ND	ND
	C3 phenanthrene 1 anthracene	30	12	ND	ND

2.2. Photosynthetic pigment contents

Photosynthesis is the process in which plants use the energy from sunlight to produce sugar, or glucose, by converting carbon dioxide into carbohydrates. However, when water is polluted, the capacity of water to dissolve gases such as carbon dioxide is negatively affected.

Overall growth and development of plants are functions of various environmental factors such as air, water, and soil (Katiyar and Dubey, 2000). The variation in leaf pigment (chlorophyll) content in plants is because of these factors. Oil pollution might be the cause of inhibition of chlorophyll synthesis since it has various metals and polycyclic hydrocarbons, thus inhibiting the enzyme necessary for synthesizing chlorophyll particles (Eller, 1977; Hope *et al.,* 1991; Keller and Lamprecht, 1995; Anthony, 2001). It is evident from the present investigation that chlorophyll and carotenoids content showed different responses to pollution. Decrease in total chlorophyll content in the leaves may be due to the alkaline condition created by dissolution of chemicals present in the oil in cell sap which is responsible for chlorophyll degradation. Total chlorophyll content of polluted leaves is lower than that of control leaves and is reported by several researchers (Somashekar *et al.,* 1999; Mandal and Mukherji, 2000; Samal and Santra, 2002).

The pattern changes in the content of chlorophyll a, chlorophyll b, and carotenoids, in *Avicennia marina* growing in Ras Mohammed area are recorded in Table (5). These data cleared significant decrease in the content chlorophyll a, chlorophyll b, and carotenoids, in *Avicennia marina*. The Most significant decrease was observed in plot (1) (1.36 ±0.07, 2.29 ±0.11 and 2.76 ±0.14 mg g^{-1}F.W.). Similar results were reported by Victor *et al.* (2002), Ye and Tam (2007) and Wang *et al.* (2011) demonstrated that oil pollution resulted in physiological damage including decreases in chlorophyll and carotenoid contents. A biotic stress, among other changes, has the ability to reduce the tissue concentrations of chlorophylls and carotenoids (Havaux, 1998; Kiani *et al.,* 2008), primarily with the production of ROS in the thylakoids (Niyogi, 1999; Reddy *et al.,* 2004). However, reports dealing with the strategies to improve the pigments contents under water stress are entirely scarce. On the other hand, chlorophyll a, chlorophyll b, and carotenoids attained the highest value (2.95±0.05, 3.89 ±0.20and 4.10 ± 0.21 mg g^{-1} F.W.) in plot (4). In addition to other factors, changes in photosynthetic pigments are of paramount importance to oil pollution. Of the two photosynthetic pigments classes, carotenoids show multifarious roles in oil pollution including light harvesting and protection from oxidative damage caused by drought. Thus, increased contents specifically of carotenoids are important for pollution.

Table (5): Photosynthetic pigments contents in the studied species *Avicennia marina*

mg g^{-1}FW	Plot 1	Plot 2	Plot 3	Plot 4
Chl.a	1.36 ±0.07	1.83±0.09	2.62 ± 0.13	2.95±0.05
Chl.b	2.29 ±0.11	2.60±0.08	2.72±0.14	3.89 ±0.20
Cart.	2.76 ±0.14	2.87±0.10	2.93±0.10	4.10 ± 0.21
Total pigment	6.41±2.1	7.3±2.8	8.27±2.2	10.94±2.2
Chl a/b	0.594±0.02	0.7±0.02	0.96±0.10	0.76±0.05

It is markedly observed from Table (5) that concentrations of chlorophyll b was greater than that of chlorophyll a in four plots; chl a/b ratio was inverted. In this connection Conklin (2001) stated that continuous oxidative assault on plants during stress has led to the presence of an enzymatic and non enzymatic antioxidant defense to counter the phenomena of oxidative stress in plants. He classified the non enzymatic plant antioxidant into two major types: (1) Ascorbic acid (AA) like scavengers, and (2) Pigments such as carotenoids.

Mechanisms are not known of precise regulations to prevent potentially destructive side reactions involving active oxygen species (AOS) that damage photosynthetic pigments and proteins (Rontein *et al.,* 2002). It is thus presumed that the role of antioxidants and carotenoids pigments in regulation photosynthetic electron transport is crucial.

2.3. Carbohydrate content

Carbohydrate changes are of a particular importance because of their direct relationship with physiological processes as photosynthesis, translocation, and respiration. Sugars are considered important metabolites in plant metabolism because provides a major source of plant respiratory energy (Harborne and Turner, 1984). Furthermore, sugar plays a number of ecological roles in plant protection against wounds, infections as well detoxification of foreign substances (Sativir *et al.,* 2000). Accumulation of sugars in different parts of plants is enhanced in response to a variety of environmental stress (Macleod and Orquodale, 1958; Gorham *et al.,* 1981; Wang *et al.,* 1996; Prado *et al.,* 2000, Gill *et al.,* 2001; Morsy, 2008).

Data presented in Table (9) and Figure (9) show variation in concentration of carbohydrates concentration in the studied plants in the four plots. The obtained results clearly show that there was significant difference in the concentration of carbohydrates between the four plots. One can observe great discrimination in the behavior of mangroves in accumulating carbohydrates. For instance plants in plot (1) recorded the lowest value (140.0 mg g^{-1}) meanwhile plants growing in plot (4).

Attained the highest value (190.4 mg g^{-1}). In this concern Tzvetkova, and Kolarov (1996) mentioned that concentration of total, soluble and starch decreased significantly in the trees from the polluted regions.

Table (9): Total Phenols, Total soluble protein and Carbohydrate of the studied species *Avicennia marina*

(mg g^{-1})	Plot 1	Plot 2	Plot 3	Plot 4
Total phenols (mg g^{-1})	1.46± 0.10	1.02± 0.10	0.69 ±0.02	1.00±0.02
Total soluble protein (mg g^{-1})	8.4±1.10	7.98±1.00	6± 0.9	5.7± 0.8
Total carbohydrate (mg g^{-1})	140.0±2.1	150.2±3.4	160.8± 4.2	190.4± 6.0

2.4. Malondialdehyde (MDA)

Estimation of malondialdehyde (MDA) amount, which is a secondary end product of polyunsaturated fatty acid oxidation, is widely used to measure the extent of lipid peroxidation as indicator of oxidative stress (Lin and Kao, 2000). Peroxidation of lipids in plant cells appears to be initiated by a number of ROS. The rate of lipid peroxidation in terms of MDA can be used as an indication to evaluate the tolerance of plants to oxidative stress as well as the sensitivity of plants to stress (Jain *et al.,* 2001).The significant increasing in malondialdehyde (MDA) contents in *Avicennia marina* growing in plot 1 (25.81±1.5 n mol^{-1} cm^{-1}) compare to *Avicennia marina* growing in plot 4 (18.06±1.3 n mol^{-1} cm^{-1}) was shown in Table (6). Our results showed that lipid peroxidation was influenced by oil pollution. Increase in MDA contents under stress was also found (Sairam and Srivastava, 2002; Tijen and Ìsmail, 2005; Wang and Han, 2007). Ye and Tam (2007) demonstrated that oil pollution resulted in physiological damage to *Aegiceras corniculatum* leaves, including increases in malondialdehyde contents. Zhang *et al.* (2007) investigated the effect of lubricating oil on germination, early growth and physiological responses of *Bruguiera gymnorrhiza* (L.), a common mangrove plant species in Hong Kong. Although germination of *B. gymnorrhiza* was not affected, early growth including height, leaf number and biomass of the oil-treated seedlings was significantly reduced while the content of free radicals and malondialdehyde (MDA), and the activity of superoxide dismutase (an anti-oxidant enzyme) increased with oil treatment.

Table (6): Malonadiadehyole contents in the studied species *Avicennia marina*

n mol^{-1} cm^{-1}	Plot 1	Plot 2	Plot 3	Plot 4
Malonaldihyde (DMA)	25.81±1.5	20.60±1.3	19.35±1.2	18.06±1.3

2.5. Enzyme activity:

The activity of different enzymes (peroxidase, catalase, and superoxidase dismutase and glutathione reductase) is recorded in Table (7). The activity of peroxidase was significantly increased under oil pollution in *Avicennia marina*. The highest value was detected in plot 1 (0.076 ± 0.013 Unite mg^{-1} min^{-1}) and lowest value was cleared in plot 4 plants (0.032±0.0009 Unite mg^{-1} min^{-1}). On the other hand, the activity of catalase, superoxide dismutase and glutathione reductase significantly decreased in *Avicennia marina* growing in plots (1) (0.0013 ± 0.0006, 0.31±0.006 and 0.40 ± 0.06 Unite mg^{-1} min^{-1}) .The highest activity of these enzymes detected in plants growing in plots 4 of the studied area of Ras Mohammed (0.009±0.005, 0.62 ± 0.031 and 0.60 ± 0.06 Unite mg^{-1} min^{-1}). Accumulation of ROS may be the consequence of disruption of the balance between their production and the antioxidative system activity, composed of enzymatic antioxidants such as catalase (CAT), peroxidases (POD) and superoxide dismutases (SOD), and non-enzymic scavengers, e.g. glutathione, carotenoids and ascorbate (Tukendorf and Rauser, 1990; Vangronsveld and Clijsters, 1994; Noctor and Foyer, 1998; Xiang and Oliver, 1998; Srivastava *et al.,* 2004). Also Ye and Tam (2007) demonstrated that oil pollution resulted in physiological damage to *Aegiceras corniculatum* leaves, including decreases in nitrate reductase, peroxidase and superoxide dismutase activities, and increases in malondialdehyde contents. Also many other plant species have been investigated in the search for novel antioxidants (Chu, 2000; Mantle *et al.,* 2000; Koleva *et al.,* 2002; Oke and Hamburger, 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species.

2.6. Amino acids:

The data in Table (8) cleared the fraction of amino acids in *Avicennia marina* growing in the different plots in Ras Mohammed.

Avicennia marina growing in plots (3&4) contained higher concentrations of aspartic acid, serine, glutamic acid, glycine, alanine, valine, leucine, lysine, threonine, isoleucine, arginine and ammonia than plots (1 & 2), while Phenylalanine detected only in plant growing in plots (3&4). Accumulation of other amino acids like glycine, serine and glutamate are known to regulate the metabolism in stressed photosynthesis tissues (Lawlor and Cornic, 2002). The amino acid threonine, isoleucine, ammonia (NH4) and arginine undetected in *Avicennia marina* plants growing in plots (1 & 2). Meanwhile proline content was increased in *Avicennia marina* plants growing in plots (1& 2) as compared to *Avicennia marina* plants growing in plots (3&4). The marked loss of protein-bound arginine in stressed Bermuda grass shoots has not been reported for other plants. This loss may reflect a preferential hydrolysis of arginine-rich protein. Such proteins are found in nuclei (Johns and Butler, 1962) and in ribosomes (Ts'o *et al.,* 1958). Stress can induce either an increase (West, 1962) or a decrease (Shah and Loomis, 1965.) in ribosomal RNA, but ribosomal proteins and nuclear proteins have not been investigated in connection with stress. However, basic nuclear and ribosomal proteins as a whole are rich in lysine as well as in arginine, and no such loss in protein bound lysine was detected as a result of stress. This could be interpreted to mean the loss of protein arginine involves the loss of some arginine-rich but lysine-poor protein. Furthermore, the loss in protein arginine may account for the observed very slight rise in free arginine. Proline attained the highest value among all amino acid in plot (1) (2.0064 $\mu g\ g^{-1}$). The higher accumulation of proline could be due to enhanced activities of ornithine aminotransferase (OAT) and proline 5-carboxylate reductase (P-5-CR), the enzymes involved in proline biosynthesis (Kohl *et al.,* 1990), as well as due to inhibition of proline oxidase, proline dehydrogenase (PDH) and proline catabolizing enzymes (Kandpal *et al.,* 1981). Free proline may be acting as a storage compound for both carbon and nitrogen during oil pollution, when both starch and protein synthesis are inhibited. Metabolic engineering studies revealed that proline is involved in reducing the photo damage in the thylakoid membranes by scavenging and or reducing the production of active oxygen species. Proline accumulation in plants is caused, not only by the activation of its degradation (Bohnert and Jenson, 1996). Proline acts as a free radicle scavenger and may be more important in overcoming stress than in acting as a simple osmolytes. Throughout this study, possible differences in nitrogen metabolism between plants growing in the four plots were sought. Under oil pollution, free proline accumulated to the highest levels in *Avicennia marina* growing in plot (1). Meanwhile plants growing in plots (3 & 4) attained the largest amounts of other free acid e.g aspartic acid. Aside from these minor observations, no differences were detected that might serve as a basis for explanation of the knowing differences in oil pollution except for proline results.

Table (7) Peroxidase, Catalase, superoxide dismutase and Glutathione reductase of the studied Species *Avicennia marina*.

Unite $mg^{-1}\ min^{-1}$	Plot 1	Plot 2	Plot 3	Plot 4
Peroxidase	0.076 ± 0.013	0.043 ± 0.016	0.033±0.001	0.032±0.0009
Catalase	0.0013 ± 0.0006	0.002 ± 0.001	0.008±0.001	0.009±0.005
Super oxide dismutase	0.31±0.006	0.43±0.01	0.44 ± 0.08	0.62 ± 0.031
Glutathione reductase	0.40 ± 0.06	0.42±0.02	0.50±0.008	0.60 ± 0.06

Table (8) Amino acids $\mu g\ g^{-1}$ of the studied species *Avicennia marina*

Amino acids $\mu g\ g^{-1}$	Plot 1	Plot 2	Plot 3	Plot 4
Aspartic acid	0.1682	0.13456	0.37184	0.4648
Serine	0.0502	0.04016	0.13864	0.1733
Glutamic acid	0.1386	0.11088	0.31944	0.3993
Glycine	0.0742	0.05936	0.06904	0.0863
Alanine	0.362	0.2896	0.31936	0.3992
Valine	0.3658	0.29264	0.24624	0.3078
Leucine	0.0918	0.07344	0.17968	0.2246
Phenylalanine	2.0399	1.63192	0	0
Lysine	0.1231	0.09848	0.1588	0.1985
Threonine	0	0	0.07712	0.0964
Isoleucine	0	0	0.04816	0.0602
NH4+	0	0	1.0332	1.2915
arginine	0	0	0.10776	0.1347
Proline	2.0064	1.60512	0.42056	0.5257

2.7. Total soluble protein

Levels of total soluble protein are shown in Table (9). Plants in plot (1) attained the highest value among all plots (8.4±1.10 $mg\ g^{-1}$). Meanwhile total soluble protein recorded the lowest one in plot (4) (5.7± 0.8 mg g-1). Many plants cope with stress by synthesizing and accumulating some compatible solutes, which are termed as osmoprotectant. Osmolytes play a major role in osmotic adjustment and also protect the cells by scavenging (ROS) reactive oxygen species (Pinhero *et al.,* 1997). A decrease in level of protein with a progressive increase in the accumulation amino acids was recorded in many plants during a biotic stress (Slatyer, 1969; Kramer, 1983; Good and Zaplachinski, 1994; Morsy, 1996 &2002). The decrease in protein content and simultaneous elevation in free amino acids under stress conditions can be explained by enhanced proteolysis, decreased protein synthesis or both (Thakur and Thakur, 1987; Roy- Macauley *et al.,* 1992; Tamura *et al.,* 2003). A variety of genes are induced by stress and functions of such gene product have been predicted from sequence homology with known proteins (Bohnert and Jenson, 1996; Shinozaki and Yamaguchi-Shinozaki, 1999).

Under biotic stress (oil pollution) is a chivied by cell and tissue specific physiological, biochemical and molecular mechanisms, which include specific gene expression and accumulation of specific proteins under pollution.

2.8. Total Phenols

It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). Total phenols play a significant role in the regulation of plant metabolic processes and overall plant growth (Lewis & Yamamoto, 1990). Our obtained data show significant increases in total phenol contents with the increase in pollution levels. Plants in plot (1) recorded the highest value of total phenol (1.46± 0.10 mg g-1). These obtained data are in good agreement with those obtained by Mohamed & Aly (2008) and El Hariri *et al.* (2010). Phenols act as a substrate for many antioxidants enzymes, so, it mitigates the stress injuries (Lewis & Yamamoto, 1990). In this connection phenol protect cells from potential oxidative damage and increase stability of cell membrane (Burguieres *et al.,* 2006). Moreover, Rivero *et al.* (2001) recorded that an accumulation of phenolic compounds in response to a biotic stress. This would be beneficial to achieve acclimatization and tolerance stress, since many kinds of plant phenolic have been considered to be the main lines of cell acclimatization against stress in plant.

2.9. Citric acid, jasmonic acid and salicylic acid

During the last 20 years increasing experimental evidence has associated organic acid metabolism with plant tolerance to environmental stress. Current knowledge shows that organic acids not only act as intermediates in carbon metabolism but also as key components in mechanisms that some plants use to cope with nutrient deficiencies, metal tolerance and plant-microbe interactions operating at the root-soil interphase.

The content of citric acid, jasmonic acid and salicylic acid in *Avicennia marina* plants at Ras Mohammed was illustrated in Table (10).

Table (10): Citric acid, jasmonic acid and salicylic acid of the studied species *Avicennia marina*

Organic acids mg g-1	Plot 1	Plot 2	Plot 3	Plot 4
Citric acid	0.44* ± 0.033	0.31±0.02	0.038 ±0.0038	0.030±0.001
Jasmonic acid	5.44* ± 0.46	3.81±0.19	1.46* ±0.37	1.02±0.05
Salicylic acid	3.36*±0.34	2.35±0.12	1.056 ±0.3008	0.74±0.04

The data reported that the oil pollution caused significant increase in the contents of citric acid, jasmonic acid and salicylic acid. The highest value was determined in *Avicennia marina* plants growing in plot (1) and the lowest value was cleared in plants growing in plot (4). The most striking result is the increase in material in response to oil pollution. The greatest increases are in the citric acid where it recorded value of 0.44± 0.033 mg g^{-1} in plot (1) compared with those attained in plot (4) 0.030±0.001 mg g^{-1}. Some plants accumulate some compatible solutes and exude various organic acids when exposed to environmental stress. These compatible solutes including citric have been suggested to be involved in stress tolerance by maintaining sufficient cell turgor for growth, thereby improving plant growth, protecting enzymes, and membranes. However, less evidence exists regarding the protective roles of organic acids under stress conditions. Here, we investigate the effects of citric acid as a component of the response to stress on plant growth and antioxidant enzyme activities. Internal citric acid concentration, induced defense mechanisms by increasing the activities of antioxidant enzymes. Based on these results, we suggested that citric acid is an important component of the stress response. Citric acid might play a positive role on stress tolerance

While the jasmonate (JA) pathway is critical for wound response, it is not the only signaling pathway mediating defense in plants. To build an optimal yet efficient defense, the different defense pathways must be capable of cross talk to fine-tune and specify responses to abiotic and biotic challenges. One of the best studied examples of JA cross talk occurs with salicylic acid (SA). SA, a hormone, mediates defense against pathogens by inducing both the expression of stress-related genes and systemic acquired resistance (SAR), in which the whole plant gains resistance to a stress (Turner *et al.,* 2002).

Conclusion

Mangrove forests are nursery and feeding ground for fishes and numerous species of epifauna and also nesting and breeding or reproduction site for sea and shorelines birds (Biagi, and Nisbet, 2006). Mangrove plays the role of wave breaker and mechanical structure seaward side, then they protect coastal area and shoreline of sever waves, sea storm and erosion. Mangroves are major producer of detritus that will contribute to offshore productivity. With continuing degradation and destruction of mangroves, there is an urgent need to conserve and restore the mangroves as defense against tidal waves, wherever muddy substrates are available and tidal flushing are regular. Human inhabitations should be permitted only behind mangrove forests but not in front of the forest. The habitat between human inhabitations and sea should be planted with suitable plant species to protect the coastal lives against natural calamities (Kathiresan and Rajendran, 2005). Mangroves have developed a complex series of physiological mechanisms to enable them to survive in a low-oxygen, high-salinity world. A major point to remember in terms of physical effects of oil spills on mangroves is that many, if not most, of these adaptations depend on unimpeded exchange with either water or air. Pneumatophores and their lenticels tend to be located in the same portions of the intertidal most heavily impacted by stranded oil. While coatings of oil can also interfere with salt exchange, the leaves and submerged roots of the mangrove responsible for mediation of salts are often located away from the tidally influenced (and most likely to be oiled) portions of the plant. These physical impacts of oil are linked to adaptive physiology of the mangrove plants, but are independent of any inherent chemical toxicity in the oil itself. The additional impact from acute or chronic toxicity of the oil would exacerbate the influence of physical smothering. Although many studies and reviews of mangroves and oil indicate that physical mechanisms are the primary means by which oil adversely affects mangroves, other reviewers and mangrove experts discount this weighting.

As we have noted, the toxicity implications from an oil spill in a mangrove area depend on a wide variety of different factors. Generally, the amount of oil reaching the mangroves and the length of time spilled oil remains near the mangroves are key variables in determining the severity of effect. Although it is stating the obvious to a spill responder that prevention is the best tool for minimizing the environmental impacts of an incident, for mangroves this is especially true. Reducing the amount of oil reaching the mangroves not only reduces the short- and long-term toxicological effects but also reduces cleanup impacts and the potential for chronic contamination. In a response, these considerations may translate into increased protection for mangroves at risk from exposure and possible use of response measures that reduce that exposure (e.g., open water countermeasures such as burning or dispersants, shoreline countermeasures such as chemical cleaners or flushing). The long-term character of many of the mangrove impacts that have been observed argues for serious consideration of such strategies.

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