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Post-spill Behavior in an Oil Contaminated Mangrove Stand *Avicennia marina* (Forssk.) Vierh in UAE.

Abstract: The effect of post-spill defoliation and the subsequent readjustment in resource allocation was investigated in relation to individual reproductive fitness of the monotypic mangrove stands *Avicennia marina* in Umm Al-Qwain Bay, northeastern United Arab Emirates. The effects of the persistence of high levels of oil hydrocarbons in the sediments after the spill on propagule dispersal, seedling recruitment, and anomalous vegetative growth forms were also studied. Growth and physiological performance of the new individuals produced from polluted and unpolluted vegetations were compared under glasshouse conditions.

Vegetative recovery of the oiled mangrove stands from post-spill massive defoliation had a negative effect on all stages of plant reproductive events including flowering, fruiting, and propagule dispersal. Persistence of toxic levels of oil hydrocarbons in the substrate has further reduced the possibility of successful establishment of the new generation in the contaminated site. A significant correlation exists between the levels of hydrocarbons in the sediments and the degree of anomalies in shoot growth of seedlings (r^2=0.862) and the newly growing pneumatophores (r^2=0.827).

Improving substrate condition by reducing levels of toxic hydrocarbons would increase the chance for better recruitment and performance of the new generation of seedlings. Active rehabilitation processes at the site may enhance the site productivity and minimize time for natural recovery.

Keywords: Defoliation, reproductive effort, seedlings recruitment, anomalous growth, oil hydrocarbons, physiological responses

سلوك أشجار الشورى (المانجروف) بعد التلوث في البقع النفطية بدولة الإمارات العربية المتحدة

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المستخلص: في هذا العمل تم دراسة تأثير تساقط أوراق نبات الشورى في فترة التي تلي التلوث بالنفط مباشرة، والتغير الناجم عنه في توزيع النبات لحصيلته الغذائية على أعضاءه المختلفة، على قدرة الأفراد التكاثرية في خليج أم القيوين في شمال شرق الإمارات العربية المتحدة. وقد تم كذلك دراسة تأثير التراكيز المرتفع للمواد الهيدروكربونية البترولية في التربة على انتشار واستقرار البادرات ودرجات التشوهات الظاهرية بها، ومقارنة معدل النمو والأداء الفسيولوجي للبادرات الجديدة الناتجة من نباتات متأثرة وغير متأثرة بالتلوث تحت ظروف الصوب الزراعية.

وقد تبين من البحث أن الاستعادة الخضرية لنباتات الشورى المعرضة للتلوث والتي تساقطت أوراقها، كان له الأثر السلبي على كل مراحل التكاثر من إزهار وإثمار وانتشار البادرات. وكذلك فان استمرار وجود تراكيز سامة من المواد الهيدروكربونية بترولية المصدر في التربة قد قلل من إمكانية نجاح الأجيال الجديدة من البادرات من البقاء والاستقرار في المناطق الملوثة. ولقد وجدت هناك علاقة معنوية بين مستويات المواد الهيدروكربونية في التربة ودرجة التشوه في البادرات والجذور التنفسية الجديدة.

ولقد خلص البحث إلى أن تحسين ظروف التربة عن طريق تقليل مستوى المواد الهيدركربونية السامة ربما يزيد من فرصة الاستقرار والأداء الفسيولوجي الجيد للأفراد الجديدة، وكذلك فان التدخل بتنظيف التربة واستزراع البادرات في المناطق المتأثرة بالتلوث يمكن أن يكون له عظيم الأثر على إنتاجية المنطقة والإسراع من الاستعادة الطبيعية للغطاء الخضري.

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Introduction

Oil spills pose a considerable threat to coastal habitat in general (Mearns, 1997) and mangrove ecosystems in particular (Burnes *et al.*, 1994). The exact effect of oil on mangroves is largely dependent on many factors such as the degree of weathering of oil after the spill, substrate aeration and the specific age classes of plants affected by the spill. Oil effect on mangroves could be physical by coating sites of gas exchange on both roots and shoots, and chemical by introducing toxic materials such as polynuclear aromatic hydrocarbons into the plant tissues (Grant *et al.,* 1993; Proffitt *et al.,* 1995 and Duke *et al.*, 1998).

In response to many environmental stresses, perennial species often divert a significant part of their resources, which are normally devoted to their reproductive structures and growth (Harper, 1977; Barbour *et al.,* 1987 and Bazzaz and Ackerly 1992), to vegetative growth. Any change in the optimum allocation of these resources normally results in a parallel change in the individual function output and fitness. During a post-spill period, recovery of the affected *Avicennia marina* trees through replacing the shed oil coated leaves would, therefore, lead to a decrease in the plant reproductive effort and subsequently the number of new individuals produced and recruited in the following season.

In January 1998, a total of 4,000 metric tons of crude oil spilled into the northeastern shores of United Arab Emirates along the Arabian Gulf and oil was discharged into the coastal area of Umm Al-Qwain Bay. The affected areas were estimated to cover about 20 km in length, composed of a monotypic mangrove population of *Avicennia marina* (Western, 1989) and some salt marsh areas. Mangrove population in the Bay is reported to be the best performing mangrove stands along the United Arab Emirates coast (El-Amry, 1998). Small islands and lagoons near and within the Bay of Umm Al-Qwain were severely affected by the spill.

The present work investigates: 1) the effect of post-spill vegetative recovery from defoliation in the oil affected mature mangroves on their reproductive effort; 2) the effect of high level of the hydrocarbons contamination in the sediments on the recruitment and growth performance of the post-spill generation; and 3) a study of the correlation between contaminants in the sediments with any abnormal growth form in both mature trees and juvenile seedlings.

Materials and Methods

1. Study Site

Umm Al-Qwain Bay (Figure 1: reproduced from Embabi, 1993) is located between 25°45′ and 25°55′ N and 55°30′ to 55°50′ E. Despite the successful attempts at removing oil from water and soil, several small islands and lagoons were severely affected by the spill. As-Sayniah is one island of the most affected areas and selected to be the polluted site under investigation. Waterfront mangrove trees on As-Sayniah Island were coated with crude oil up to 120 cm (average tidal range), while small age classes of mangrove (1 to 4 years) were completely covered. Al-Qirm, on the other hand, is one of the biggest islands in the Bay, which has not been affected by the spill and is considered to be the reference (non-polluted) area for comparison. *Avicennia marina* (Forssk.) Vierh is the only existing mangrove species in the Bay (Western, 1989). Sites were visited regularly starting one month after the spill (February 1998) till the end of the fruiting season and seedling establishment (December 1998).

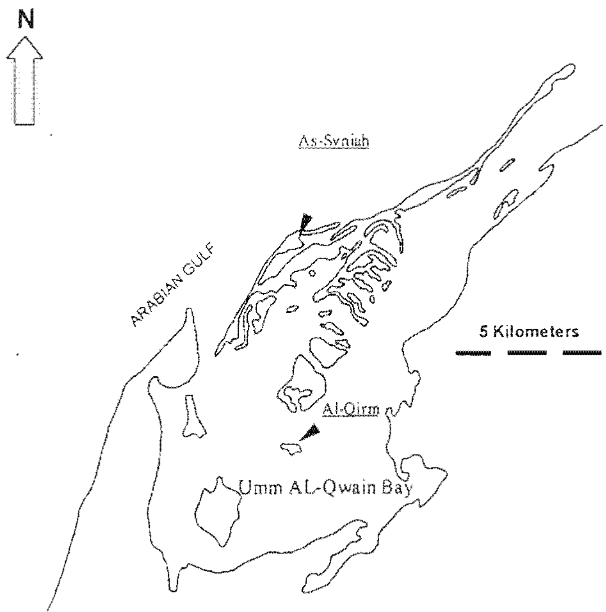

Figure 1: Locality map of Umm-Al-Qwain Bay, United Arab Emirates, showing the two study sites As-Sayniah (the oil affected) and Al-Qirm (the control) islands. (Adopted from Embabi, 1993).

2. Chemical Analysis

Sediment samples were collected from six randomly selected quadrates (5m x 5m) in both polluted and non-polluted sites (As-Sayniah & Al-Qirm) by means of a coring device equipped with a 22 mm diameter polycarbonate core liner to a depth of 10 cm. Soil samples were collected at the period after affected trees began defoliation (February 1998). Oiled and non-oiled leaves from mature mangrove trees were sampled in the same areas where soil samples were taken. Soil and leaf samples were collected in proper glass containers and kept frozen till laboratory analysis. Percent dry weight for soil and tissue samples was measured from separate portions of these samples. Known fresh weights of soil or plant tissue samples (10 g) were mixed with the equal weights of anhydrous Na_2SO_4 and extracted in a Soxhlet extractor apparatus for 24 hrs using 300ml of acetone/hexane (1:1) (V/V), as an extraction solvent (US EPA, 2000). After extraction was completed, extracts were concentrated to 1ml using a rotary evaporator and then analyzed for total hydrocarbon and polynuclear aromatic hydrocarbons using GC/FID. 10 mL of extracts were injected into a 30m x 0.25 mm SE-54 fused silica column (US EPA, 2000). After extraction was completed, extracts were concentrated to 1ml using a rotary evaporator. Aliquots of 10ml extract were injected into a GC fitted with PTE 5, 30 m long x 0.32mm ID x 0.25mm film thickness, capillary column with 5% diphenyl/95% dimethyl siloxane bonded phase. The GC was fitted with a flame ionization detector (FID). Aliquots of 1ml extract were injected into a GC (HP5890, Series II, Hewlett Packard, USA) fitted with PTE 5, 30 m long x 0.32mm ID x 0.25 mm film thickness, capillary column (Supelco, USA) with 5% diphenyl/95% dimethyl siloxane bonded phase. The GC was fitted with a flame ionization detector (FID). The GC temperature program was from 35°C (4min) to 310°C (5min) at 1°C/min. The injector was maintained at 250°C. Helium was used as a carrier gas at a constant flow rate of 1ml/min. The FID was operating at 350°C with hydrogen as a fuel gas (30ml/min), air as an oxidizing gas (250ml/min) and nitrogen as a make-up gas (30ml/min).

3. Measured Parameters

All ecological measurements were taken from the above described six randomly selected quadrates (5m x 5m) in both polluted and non-polluted sites (As-Sayniah & Al-Qirm). Six mature trees within these quadrates of the same height and average age class were tagged on each site.

Post-spill defoliation measurements were based on the percentage of leaves fallen (indicated by their leaf scars) to the total number of leaves on 20-sampled branches per tree. Leaf area index and canopy cover were not considered as an appropriate measure for defoliation because of the nature of the stand and the non-uniform distribution of the affected mature trees.

Litterfall was measured as described by Proctor (1983, 1984) using trap nets (mesh type II = 2cm x 2cm) to fit the small size of the leaves and any falling parts from the trees.

Reproductive effort was measured during the flowering and fruiting of the mangrove *Avicennia marina* (from April till October) as a percent of the weight of all reproductive parts (flower buds, flowers and fruits) to the total vegetative weight for 20 branches for the six trees on each site.

The number of propagules dispersed was calculated as the number of propagules per m^2. Successfully recruited and established viable seedlings were counted and values were expressed as the number of seedlings recruited per m^2.

All forms of abnormalities in both seedling shoots and newly growing pneumatophores were recorded in the previously selected six quadrates (5m x 5m) on both sites. Values are given as a percentage of the number of total individuals recorded in every quadrate.

4. Glasshouse study

Establishment and growth performance of post-spill produced propagules (from As-Sayniah site) was compared with that of the unaffected population (from Al-Qirm site) under improved substrate conditions in a glasshouse. A total of 150 propagules were collected from each site (September, 1998) and followed at different stages of development from germination up to three month old seedlings. Both lots of propagules were grown under glasshouse conditions at an average temperature of 30±2°C and relative humidity of 40±5 % and photosynthetic active radiance (PAR) > 400 mmol m^{-2} s^{-1}. Propagules were grown on a peat moss/sand mix (1:1) supplemented with (NPK) fertilizers and irrigated with 25% seawater (Youssef, 1997). Growth parameters were measured on 20 randomly selected seedlings from each group. Differences in transpiration rate ($\mu g.m^{-2}.s^{-1}$), stomatal conductance ($s.cm^{-1}$) and leaf-to-air temperature differential (°C), (Youssef and Saenger 1998) among the seedlings two groups were measured once at a fixed time of the day using a steady state Porometer (LI-COR 1600, NE -USA).

5- Statistical Analysis

Data were subject to one-way analysis of variance ANOVA, and regression analysis using the statistical software program Systat 5.2 (SYSTAT, Inc., Evanston,IL).

Results and Discussion

I- Oil Hydrocarbons Contaminants

Substantial quantities of total petroleum hydrocarbons (THs) were recorded in both contaminated sediments and leaves (Table 1 & 2).

Table 1: Total oil hydrocarbons (THs), petroleum hydrocarbons in diesel range (DRO), polynuclear aromatic hydrocarbons (PAHs) and total aliphatic hydrocarbons (TAHs) in mangrove sediments collected from oil polluted (As-Sayniah) and non-polluted (Al-Qirm) islands in Umm-Al-Qwain Bay. (**) & (***); values are significant at *P>0.01* &. *P>0.001* respectively. ND; not detected.

Parameters	Oiled Site As-Sayniah Island	Non-Oiled Site Al-Qirm Island
Total Hydrocarbons (THs) (ppt)	** 83.400 ± 011.08	0.226 ± 0.113
Petroleum Hydrocarbons in Diesel Range (DRO)(ppt)	*** 04.570 ± 01.616	0.047 ± 0.013
Polynuclear Aromatic Hydrocarbons (PAHs)(ppm)	*** 593.0 ± 12.784	ND
Total Aliphatic Hydrocarbons (TAHs)(ppm)	*** 34.00 ± 00.012	ND

Quantities of petroleum hydrocarbons in the diesel range (DRO) were relatively low. The type of hydrocarbons remaining in the sediments and plants after one month of weathering are essentially heavy fractions of crude oil (e.g. waxes and asphaltenes). Polyneuclear aromatic hydrocarbons (PAHs) in all contaminated samples confirm the source of contamination as crude oil. Aliphatic hydrocarbon (TAHs) up to *n*-hexacontane were present only in oil affected sediment samples. As shown in Table (1) THs, were significantly higher (*P<0.01*) in the sediment samples from affected sites one month after the spill (February 1998). Both DRO and PAHs were also significantly higher in the oiled site (*P<0.001*). The high concentration of PAHs recorded is thought to be the outcome of covering oil-contaminated sediments with a thick layer of sand to absorb the oil (a practice that was recommend to locals by an unknown source). In addition, several authors reported that in coastal areas, oil-derived hydrocarbons might remain in the sediments for several years after the spill (see review by Lee, 1980). Oil volatile components normally disappear within a few hours, particularly under arid conditions. However, natural photochemical degradation of certain oil components normally result in polar molecules having a higher water solubility and thereby increasing their immediate potential toxicity (Lee, 1980). Photodegradation of the aromatic fraction of the crude oil under investigation was evident from the high levels of fluorenone and benzoic acid in the PAHs fraction (data not shown).

Samples of old leaves from the mature vegetation (non-oiled leaves) and the newly growing leaves replacing the defoliated ones were analyzed for oil hydrocarbons (Table 2). THs were low and in some cases undetected in the newly growing leaves compared with the old ones. However, this data may also indicate that many different oil components can reach leaf tissues in a water-soluble form. The natural background of THs in an unpolluted coastal environment is about 1-30 ppb, while toxic levels of most hydrocarbons including active water soluble fractions containing aromatics should be greater than 0.1ppm (Oppenheimer, 1980). Values given in the present studies indicate that all fractions of the total hydrocarbons, except aliphatics, remained at toxic levels when sampling took place.

Table 2: Total oil hydrocarbons (THs), petroleum hydrocarbons in diesel range (DRO), polynuclear aromatic hydrocarbons (PAHs) and total aliphatic hydrocarbons (TAHs) in old leaves (not physically coated with oil: before defoliation) new leaves (after defoliation) in oil affected mangrove trees *Avicennia marina* in As-Sayniah island, Umm-Al-Qwain Bay. (*), (**) & (***); values are significant at *P>0.05*, *P>0.01* &. *P>0.001* respectively. NS; not significantly different. ND; not detected.

Parameters	New leaves After defoliation	Non-oiled leaves Before defoliation
Total Hydrocarbon (THs) (ppt)	5.2 ± 0.213	* 09.8 ± 0.40
Petroleum Hydrocarbons in Diesel Range (DRO) (ppt)	ND	*** 0.27 ± 0.18
Polyneuclear Aromatic Hydrocarbons (PAHs) (ppm)	28.9 ± 2.600	**37.10 ± 3.11

II- Post-spill recovery and Reproductive efforts of the mature trees.

In response to the spill and the physical coating of oil to leaves below the tidal range in As-Sayniah, the average litterfall weight per unit area and percentage defoliation were significantly higher (*P>0.001 & P>0.05* respectively) than that in the control site (Al-Qirm) (Figures 2A and 2B). Litterfall weights were 520 g/m^{-2} in As-Sayniah and 110 g/m^{-2} in Al-Qirm respectively. Percentage defoliation in As-Sayniah was 42%, compared to 21 % in Al-Qirm. Defoliation is possibly due to severe stress on leaves coated with oil or a mechanism by which plants avoid being loaded with the infiltrating toxic hydrocarbons through the oil-coated leaves. Analysis of polynuclear aromatic hydrocarbons as shown in Table (2), indicates that leaf shedding in itself may be an effective way of localizing these toxic materials and preventing them from reaching the newly growing leaves.

Plants have a limited supply of some critical resources, which are normally divided between growth, maintenance and reproduction (Barbour *et al*., 1987 and Bazzaz and Ackerly, 1992). These functions are assumed to be mutually exclusive, such that allocation to one function causes a decrease in allocations to other functions (Wilson, 1983). Under different environmental stress conditions, which lead to a shift in resource allocation, a decrease in the plant function output or plant fitness would occur.

The reproductive efforts significantly decreased (*P>0.001*) from 26% in the control site to 9% in the unpolluted site (Fig. 2C). Such change in the proportional investment in reproduction versus vegetative growth is indicative of the stress level of the vegetation (Freeman *et al.* 1997). Furthermore, plants growing under arid environments (as in the present case) should normally exhibit a high reproductive effort (Boaza *et al.,* 1994). During the flowering and fruiting season (September - October), resource diversion toward the vegetative growth was evident in the output of all reproductive stages of the affected plants. The average fresh weight of fruit produced by oiled trees, for example, was almost half that produced by non-oiled trees (2.2 to 4.2 g/fruit, *P>0.001* respectively) (Figure 2D). The percent of propagules dispersed at the unpolluted site was almost six times that of the polluted site *(P>0.001)* (Figure 2E). The remarkably low dispersal of the polluted site is a direct outcome of the low number of fruits produced during the reproductive season. Furthermore, the number of propagules recruited (only viable seedlings were considered) in the polluted site was only 17% of the number of seedlings recruited in the unpolluted site (*P>0.001*) (Figure 2F).

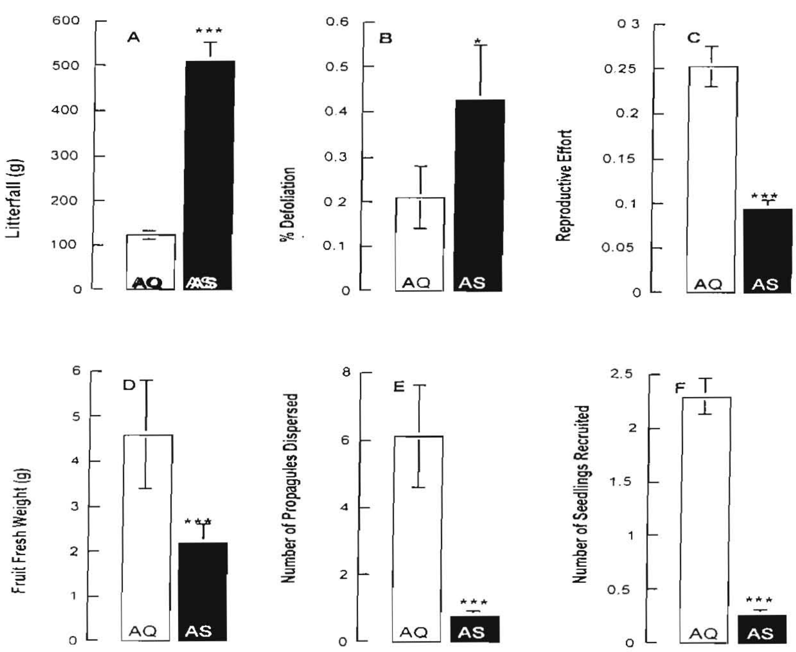

Figure 2: Differences in A- Litterfall, B- Percentage defoliation, C- Reproductive effort D- Average fruits fresh weight, E- Number of propagules dispersed per square meter, and F- Number of propagules recruited per square meter between the mangrove *Avicennia marina* in oil polluted (As-Sayniah: AS) and non-polluted (Al-Qirm: AQ) islands, Umm-Al-Qwain Bay. (*), (**) & (***); values are significant at *P>0.05, P>0.01* &. *P>0.001* respectively.

III- Seedlings performance under controlled conditions.

Success of seedling recruitment under field conditions is controlled by many factors such as propagule viability, number of predators and substrate condition. Accordingly, propagules were transferred to a suitable substrate and grown for three months under glasshouse conditions in order to highlight the effect of contaminated substrate as a limiting factor for recruitment and seedling performance. Both groups of propagules, collected from oiled and non-oiled sites, showed no significant difference in percentage germination (Figure 3A). As an indirect measure of seedlings' abilities for resource acquisition, growth parameters, including the fresh and dry weight of total seedlings and total leaf area per seedling were compared for both groups of propagules, collected from oiled and non-oiled sites. There was a significant difference in all of the recorded parameters (except for the dry weight of seedlings, significant at $P> 0.05$) (See Figures 3B, C & D). As shown in Table (3), the two lots of seedlings showed no significant difference in utilizing the available resources when grown on non-polluted substrate. Similar evidence on the effect of oil and growth of other mangrove species (*Rhizophora mangle*) was studied on experimentally oiled peaty mangrove soil by Scherrer *et al.*, (1989).

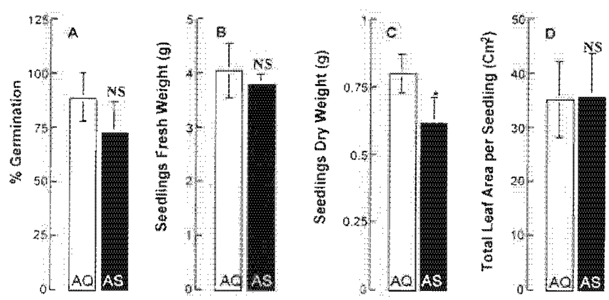

Figure 3: Growth parameters, including A-Percentage germination. B-Total seedling fresh (g), C- Total seedling dry weight (g), and D- Total leaf area per seedling (Cm^2) of propagules of the mangrove *Avicennia marina* grown under glasshouse conditions. Propagules were collected from oil polluted site (As-Sayniah: AS) and non-polluted (Al-Qirm: AQ) islands, Umm-Al-Qwain Bay. (*), values are significant at $P>0.05$. NS; not significantly different.

In addition, physiological performance was measured through individual ability to maintain appreciable conductance to air while maintaining leaf temperature within a range through evaporative cooling by transpiration. Once again, the two plant groups were identical despite the difference in average fruit size and weight from which they originated (Table 3).

IV. Anomalous Growth Forms

Several authors have reported observations on anomalous root growth of mangroves in response to oil spills (e.g. Snedaker *et al.*, 1981 and Getter *et al.*, 1984), including in the Gulf region (e.g. Spooner, 1970 and Böer, 1993). In the present work the correlation between levels of hydrocarbons in the sediments and the degree of anomalies in the vegetation was studied through regression analysis (Figure 4). Anomalous root growth forms in the growing pneumatophores of the mature stand were only observed seven months after the spill event (August-September 1998). Multiple branching, to two or three branches, of this respiratory negative geotropic root represented 11.1% of the total number of pneumatophores considered at the polluted site. In the non-polluted site anomalies in pneumatophores represented only 2.3%. Although branching pneumatophores do exist within non-oiled mangrove stands, their higher percentage in the polluted site seems to be correlated to the intensity of the spill ($r^2=0.827$).

Within seedlings recruited in previous growing seasons, the percent of anomalous shoot growth forms was 12% at the affected site, compared with 1.8% at the unaffected site. Multiple branching of the shoot to two or three branches, which is uncommon in healthy seedlings, represented these anomalous growth forms. Such loss in the epical dominance and the subsequent multiple branching is probably due to the damage caused to the terminal growing buds of the seedlings during the spill ($r^2=0.862$).

Table 3: Physiological performance parameters for seedlings of the mangrove *Avicennia marina* collected from oil polluted (As-Sayniah) and unpolluted (Al-Qirm) islands and grown for 3 months under controlled conditions. Stomatal conductance (s cm^{-1}), transpiration rate (mg m^{-2} s^{-1}), and leaf -to-air temperature differential (°C).

Parameters	Plant Source	
	Oiled mother tree	Non-oiled Mother tree
Stomatal Conductance	0.875 ± 0.22	0.948 ± 0.24 (NS)
Transpiration Rate	17.66 ± 3.84	17.940 ± 3.88 (NS)
Temperature Differential	1.27 ± 0.25	1.140 ± 0.22 (NS)

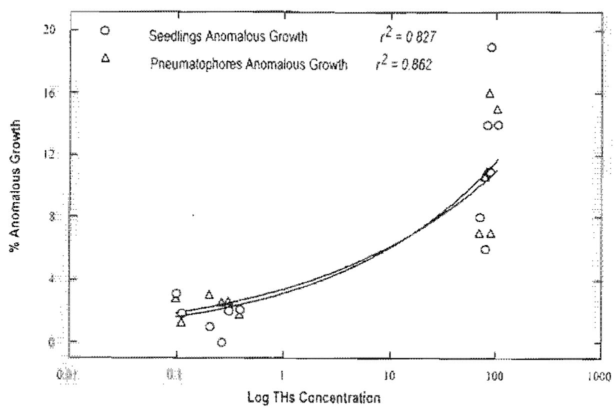

Figure 4: Relationship between levels of hydrocarbons in the sediments and levels of anomalous growth forms of the mangrove *Avicennia marina* in oil polluted site ((As-Sayniah: AS) and non-polluted (Al-Qirm: AQ) islands, Umm-Al-Qwain Bay. (r^2) is the regression coefficient.

Acknowledgments

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