

T Youssef

# Physiological Responses of *Avicennia marina* Seedlings to the Phytotoxic Effects of the Water-Soluble Fraction of Light Arabian Crude Oil

**Abstract:** Stomatal behavior, growth performance and accumulation of polynuclear aromatic hydrocarbons (PAHs) were evaluated in seedlings of the mangrove *Avicennia marina* (Forssk.) Vierh, treated with the water-soluble fraction (WSF) of Abu-Dhabi light Arabian crude oil through foliar spraying or soil application. Irregular stomatal behavior and weak stomatal control over transpiration were observed during the first 24 hours, where stomatal resistance of plants sprayed with 150 and 300 (g PAHs plant<sup>-1</sup>) was significantly lower than that of the control plants. After six weeks, all treated plants showed no significant difference in their relative growth rate (RGR) or in the net assimilation rate (NAR) compared with the control plants. Tri-aromatic hydrocarbons were the most accumulated in tissues of the treated plants. Penta- and hexa-aromatics, on the other hand, were undetectable in the WSF and consequently in the treated plants. A linear relationship was observed between the dose applied to plants and the amounts of tissue accumulated PAHs ( $r^2=0.515$  for soil application and  $r^2=0.984$  for foliar spray). In plants sprayed with 300 (g PAHs plant<sup>-1</sup>), the total PAHs accumulated were more than that accumulated in plants treated through soil application. These findings suggest that:

1. Aqueous extraction of crude oil tends to signify the percentage of the low molecular weight PAHs, e.g. naphthalene to the total PAHs.
2. Disturbed stomatal behavior in the first day of the treatment may be due to the venting of the volatile low molecular weight aromatic hydrocarbons (e.g. benzene, toluene, and xylenes) through the stomata.
3. Uptake of water-soluble hydrocarbons by plants is equally possible through both the root system and the foliage.

The ecological implications of these findings are discussed in relation to oil pollution of mangrove stands under field conditions.

**Keywords:** Desertification, Population growth, Urbanization, Ecological Degradation, Environmental Policies.

Tarek Youssef

Department of Biology, United Arab Emirates University,  
P.O. Box 17551, Al-Ain, United Arab Emirates

Fax: +97137516726

Tel: +971504480864

Email: tyoussef@emirates.net.ae

التأثير الفسيولوجي للمستخلص الذائب لزيت البترول العربي الخفيف على بادرات نباتات الشوري (المانجروف) طارق يوسف

المستخلص: تناول البحث بالدراسة سلوك الثغور ومعدلات النمو وتراكم المركبات الهيدروكربونية الحلقيّة في بادرات أشجار الشوري بعد معالجتها بالمستخلص الذائب لزيت البترول العربي الخفيف. ولقد وجد أن البادرات المعالجة أقل قدرة على التحكم في معدل النتح من خلال الثغور، ولم يكن هناك أي إختلاف بين المعاملات في معدل النمو النسبي، ولا معدل التمثيل بعد ستة أسابيع من المعالجة.

تبين أن الهيدروكربونات البترولية، ثلاثية الحلقة، أكثر تراكمًا من رباعيات أو خماسيات الحلقات. وأن هناك علاقة خطية معنوية بين كميات الهيدروكربونات المتراكمة والكمية المعالج بها النباتات من خلال الرش الورقي. ويمكن أن تدلل هذه النتائج على أن المستخلصات المائية الذائبة للبترول الخام تزيد من فرص التعرض إلى الهيدروكربونات قليلة الحلقات. كما يمكن أن تدلل على أن الخل في سلول الثغور مع المعالجة ربما يعزي إلى خروج بعض المركبات الحلقيّة الصغيرة منها مثل البنزين والبولينين والزايلين. إضافة إلى أن إمتصاص الهيدروكربونات الذائبة ممكناً من خلال الورقة والجذور.

كلمات مدخلية: التصحر، النمو السكاني، التحضر، إنحلال بيئي، سياسات بيئية.

## Introduction

Mangrove is one of the most potentially vulnerable of the intertidal communities to oil pollution (den Hartog, 1984). In oil contaminated areas, mangrove seedlings, unlike the mature stand, may become fully submerged with the WSF of oil several hours a day during the tidal cycle. Thereby, they are likely to accumulate more PAHs than other age groups of heights above watertable. As mangrove is one of the most important primary producers in the coastal habitat, this particular age group of seedlings would participate more than the rest of the vegetation in introducing PAHs or their metabolic transformation products to marine food webs.

Several authors have studied the effect of different types of crude oil on various mangrove species under field conditions e.g. (Getter *et al.* 1985; Scherrer *et al.* 1989 and Klekowski *et al.* 1994 a&b) and laboratory conditions (Getter *et al.* 1983;

Teas *et al.* 1985; Knap, 1987; Grant *et al.* 1993; Proffitt *et al.* 1995; Proffitt and Devlin, 1998 and Duke *et al.* 1998). Responses of mangroves to oil treatment vary depending on the type of the crude (Garrity *et al.* 1994), the degree of weathering (Duke *et al.* 1998), and many other environmental factors (Proffitt *et al.* 1995). These responses were previously studied by (Snedaker *et al.* 1981, Getter *et al.* 1985, Page *et al.* 1985, Bóer 1993, Klekowski *et al.* 1994 (a & b) and Duke *et al.* 1998).

Crude oil is a mixture of thousands of organic compounds, of which 75% (w/w) are hydrocarbons (Ralph and Burchett, 1998). Low molecular weight aromatic hydrocarbons (e.g. benzene, toluene, xylenes, etc.) are considered the initial source of biological toxicity caused by unweathered crudes (Grant *et al.* 1993 and Ralph and Burchett, 1998). However, these compounds are extremely volatile and their effect may become negligible within a few hours (Durako *et al.* 1993). Polynuclear aromatic hydrocarbons (PAHs), on the other hand, are another important group of hydrocarbons, known to be mutagenic to mangroves (Klekowski *et al.* 1994 a&b) and phytotoxic to other plants e.g. seagrasses (Durako *et al.* 1993 and Ralph and Burchett 1998).

As crude oil loses most of its toxic components by evaporation and dissolution in water during the weathering process, studies on the phytotoxicity of the water-soluble fraction (WSF) of crude oil is warranted. None of the available reports, however, considered the physiological responses of mangroves to the phytotoxic effects mediated by WSF of oil without plants being in physical contact with the crude.

Despite the hydrophobic nature of PAHs, they are considered the major component causing most of the biological damaging effects after any spill (Lee and Page, 1997). On the other hand, *Avicennia marina* is one of the most sensitive mangrove species to oil, particularly light crude (Getter *et al.* 1985, Duke *et al.* 1998). The present paper investigates the physiological responses of *Avicennia marina* seedlings to the water extract of light Arabian crude oil and the differential affinities of its toxic PAH component to accumulate within the seedling tissues.

## Materials and methods

A total of 250 viviparous propagules of the mangrove *Avicennia marina* (Forsk.) Vierh., were collected from healthy trees on the Abu-Dhabi coast (54° 50' E and 24° 50' N). Propagules of similar dimensions were raised as described in (Youssef

and Saenger, 1998). After 4 weeks successfully raised seedlings were subsequently transferred individually to suitable sized pots containing the same type of substrate saturated with 25% seawater. Seedlings were then left to grow for another four weeks under glasshouse conditions at an average temperature of 30±2°C, relative humidity of 40±5%, and photosynthetic active radiance (PAR) > 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

Approximately 6.6 l of Abu Dhabi light Arabian crude oil (ADCO-UAE) was used in the present study. The protocol adopted for oil extraction is modified from that described in Ralph and Burchett (1998). Oil/seawater mixtures (1:1)(v/v) were left on a shaker for 24 hours to ensure maximum solubility into the aqueous phase. Mixtures were then allowed to settle for 1h before removing the oil layer by filtration through cotton wool. Aqueous extracts were then added together to form the stock solution of WSF. Both crude oil and the water extract (WSF) were analyzed for PAHs.

One hundred and fifteen seedlings of similar dimensions were selected for the current experiment. Ten seedlings were harvested at the beginning of the experiment to estimate the initial average dry weight and average leaf area. The remaining seedlings were randomly assigned for the following treatments (7 treatments each containing 15 seedlings); control - 0  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$ , 75  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$  supplied to plants through soil application, 75  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$  supplied through foliar spraying, 150  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$ -soil application, 150  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$ -foliar spray, 300  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$ -soil application, and 300  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$ -foliar spray. Equivalent volumes to the above concentration were calculated from Table 1 as shown in Table 2.

Plants were treated only once with WSF in the first day of the experiment (at midnight local time) and left overnight. Differences in transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ ), stomatal resistance ( $\text{s cm}^{-1}$ ) were measured on 10 seedlings per treatment in the early morning of the following day and continued for 48 hours (after this period all readings appeared insignificantly different, data not shown). Measurements were made at fixed times of the day (6:00, 12:00, 15:00, 18:00 local time) using a steady state Porometer (LI-COR 1600, NE -USA). Environmental parameters (quantum flux density, air temperature, and relative humidity) were recorded over the 48 hours. After 48 hours only 5 seedlings were harvested for latter chemical analysis for PAHs.

**Table 1.** Concentration of parent PAH compounds ranging from di- to hexa- aromatic hydrocarbons in Abu-Dhabi light Arabian crude oil (LAC) and its aqueous extract (WSF) after a 24h-extraction period. The percentage extractable from crude oil into the aqueous phase is calculated as a ratio of the original concentrations of each compound in the crude. Total PAHs is the sum of all listed compounds. ND = not detectable.

No. of Rings	Compound	LAC ( $\mu\text{g ml}^{-1}$ )	WSF ( $\mu\text{g ml}^{-1}$ )	Extractable %
2	Naphthalene	132	0.81	0.6136
3	Acenaphthalene	550	0.17	0.0309
3	Acenaphthene	592	0.24	0.0405
3	Fluorene	5707	0.04	0.0007
3	Phenanthrene	5881	0.45	0.0077
3	Anthracene	5880	0.45	0.0077
4	Fluoroanthene	2127	0.47	0.0221
4	Pyrene	2127	0.47	0.0221
4	Benzo(a) anthracene	432	0.35	0.0810
4	Chrysene	ND	ND	ND
5	Benzo(b) fluoranthene	294	ND	0.0000
5	Benzo(k) fluoranthene	ND	ND	ND
5	Benzopyrene	ND	ND	ND
5	Dibenzo (a-h) anthracene	ND	ND	ND
6	Indeno (1,2,3 cd) pyrene	ND	ND	ND
6	Benzo(ghi) pyrene	311	ND	0.0000
<b>Total PAHs</b>		<b>24033</b>	<b>3.45</b>	<b>0.0144</b>

**Table 2.** Relative mixing volumes of the water-soluble fraction of crude oil (WSF) and 25% seawater in preparation of volumes equivalent to different PAH doses applied to *Avicennia marina* seedlings as foliar spray (FS) or through soil application (SA).

Treatment	WSF* (ml plant <sup>-1</sup> )		25% Seawater (ml plant <sup>-1</sup> )		
	SA	FS	SA	FS	
Control	SA	0.00	0.003	00.00	0.00
	FS	0.00	0.00	0.000	300.00
75 $\mu\text{g/plant}$	SA	21.74	0.00	278.26	0.00
	FS	0.00	21.74	0.00	278.26
150 $\mu\text{g/plant}$	SA	43.48	0.00	256.52	0.00
	FS	0.00	43.48	0.00	256.52
300 $\mu\text{g/plant}$	SA	86.96	0.00	213.04	0.00
	FS	0.00	86.96	0.00	213.04

The remaining seedlings (10 plants per treatment) were left to grow for 6 weeks before being harvested (at age 14 weeks old) for determining their relative growth rate and net assimilation rate. Leaf areas for all harvested plants were recorded. Materials were then oven-dried at 80°C for constant dry weights. Relative growth rates were calculated as described in (Ball and Pidsley, 1995). Crude oil and its water extract were analyzed for PAHs. Water samples were extracted as

described in the (US-EPA,1986). Aliquots of 1 $\mu\text{l}$  extract were injected into a GC (HP5890, Series II, Hewlet Pakard, USA) fitted with PTE 5, 30 m long x 0.32 mm ID x 0.25 (m 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 (5 min) at 1°C min<sup>-1</sup>. The injector was maintained at 250°C. Helium was used as carrier

gas at a constant flow rate of  $1\text{ ml min}^{-1}$ . The FID was operating at  $350^\circ\text{C}$  with hydrogen as a fuel gas ( $30\text{ ml min}^{-1}$ ), air as oxidizing gas ( $250\text{ ml min}^{-1}$ ) and nitrogen as make-up gas ( $30\text{ ml min}^{-1}$ ).

Similarly,  $0.2\ \mu\text{l}$  aliquots of the crude were injected into a GC fitted with a Petrocol DH 50.2,  $50\text{ m long} \times 0.20\text{ mm ID} \times 0.50\ \mu\text{m}$  film thickness, narrow bore fused silica capillary column (Supelco, USA) with polydimethylsiloxane as bonded phase. The GC was fitted with FID. All test conditions were similar to that of water samples as described above.

Plant materials (5 plants per treatment) were harvested into proper glass containers and kept frozen till laboratory analysis. Percent dry weight was measured from separate samples. Fresh weights of plant tissue samples were mixed with the equal weights of anhydrous  $\text{Na}_2\text{SO}_4$  and extracted in a Soxhlet extractor apparatus for 24 hours using  $300\text{ ml}$  of acetone/hexane (1:1) (V/V), as extraction solvent (US-EPA 1996). Extracts were concentrated to  $1\text{ ml}$  using a rotary evaporator. Aliquots of  $10\ \mu\text{l}$  extract were injected into a GC fitted with PTE 5,  $30\text{ m long} \times 0.32\text{ mm ID} \times 0.25\ \mu\text{m}$  film thickness, capillary column with 5 % diphenyl/95 % dimethyl siloxane bonded phase. The GC was fitted with a flame ionization detector (FID). The run conditions were similar to that described above.

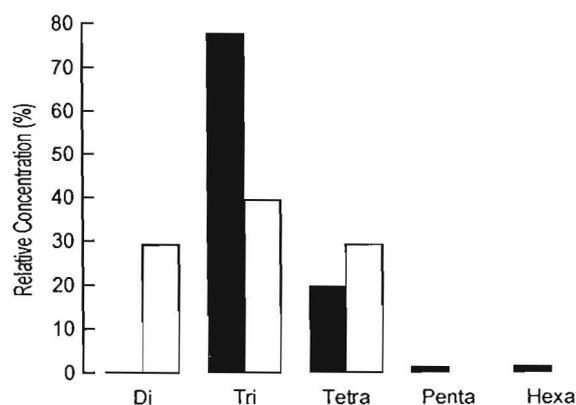
### Statistical Analysis

Stomatal behavior and growth characteristics data were subject to two-way analysis of variance ANOVA, using the statistical software Systat 5.2 (SYSTAT, Inc., Evanston, IL). Regression analysis of PAH dose-accumulation relationships were graphically approached using CA-cricket graph III software (Computer Association Corporation).

### Results

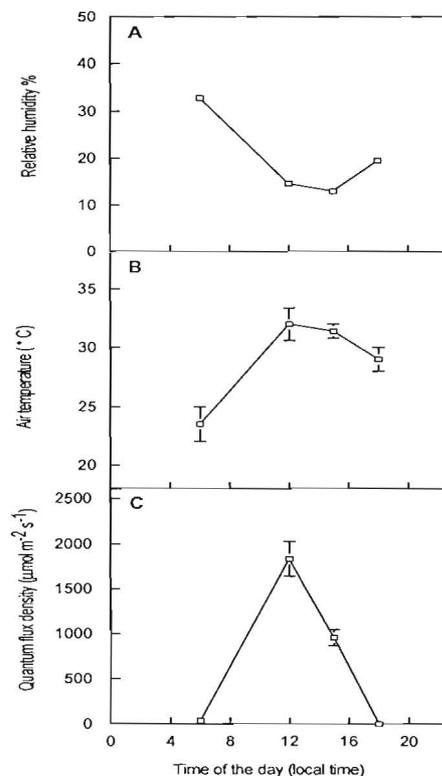
The light crude oil content of PAHs is generally high. In the present case the PAH content reached a total of  $24033\ \mu\text{g ml}^{-1}$ . Tri-aromatics were the most dominant fractions (See Table 1) representing 77.4% of the total PAH content in the crude (Fig. 1).

The percentage extractable in aqueous media of these hydrocarbons varies considerably depending on the degree of aromaticity. Naphthalene, the smallest of the PAHs, reached 0.6136 % of the total naphthalene content of the crude oil. On the other hand, benzo(ghi)pyrene, a hexa-aromatic, was undetectable in the WSF (see, Table 1, Fig. 1).



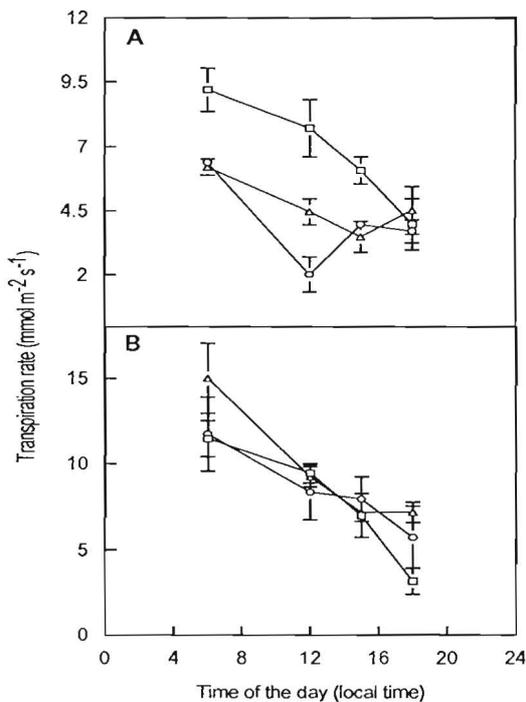
**Figure 1.** Relative concentrations of different groups of PAHs (Di-, tri-, tetra-, penta-, and hexa-aromatic hydrocarbons) in light Arabian crude and its water extract (WSF). The relative concentration of every aromatic group is given as a percentage of the total PAHs. (□) water extract (■) crude light oil.

Transpiration rate and stomatal resistance were recorded for all plants nearly 6 hours after the treatment started. Figure (2) shows the environmental conditions under which these measurements were made.



**Figure 2.** Environmental parameters (Quantum flux density, Air temperature, and Relative humidity) under which stomatal resistance and transpiration rate were measured during the first 48 hours of the experiment. Values are mean  $\pm$  SE,  $n = 70$ .

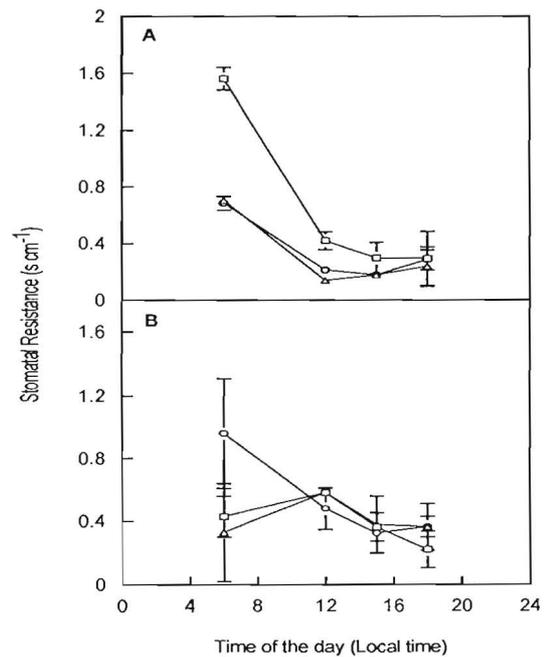
There were no clear differences in both transpiration rate and stomatal resistance between the control plants and plants treated with 75  $\mu\text{g}$  PAHs plant<sup>-1</sup> either by soil application or foliar spray. Data presented in Figure 3 and 4 have been restricted to compare plants treated with 150, and 300  $\mu\text{g}$  PAHs plant<sup>-1</sup> foliar spray with the control over 48 hours. The rate of transpiration of the control plants was significantly higher ( $9.195 \pm 0.833$  mmol m<sup>-2</sup> s<sup>-1</sup>,  $P < 0.05$ ) than the treated plants throughout the first 24 hours except at sunset. At mid-day of the first 24 hours (temperature  $32 \pm 1.4$  °C), the transpiration rate of plants treated with 300 (g PAHs plant<sup>-1</sup>) ( $2.01 \pm 0.7$  mmol m<sup>-2</sup> s<sup>-1</sup>) was significantly lower ( $P < 0.05$ ) than that of plants treated with the 150  $\mu\text{g}$  PAHs plant<sup>-1</sup> (Fig. 3A). During the second day, an overall reduction in transpiration rate was observed, with no significant differences between treatments (Fig. 3B).



**Figure 3.** Transpiration rate of *Avicennia marina* seedlings treated with the WSF of light crude oil over 48 hours. (□); control plants, (Δ); Plants sprayed with volumes of WSF equivalent to 150 (g PAHs plant<sup>-1</sup>, and (○); Plants sprayed with volumes of WSF equivalent to 300  $\mu\text{g}$  PAHs plant<sup>-1</sup> (A); measurements made during day 1, (B); measurements made during day 2. Values are mean  $\pm$  SE, n=10.

Stomatal resistance and transpiration showed opposite trends during the measurement period (48 hours). Plants treated with PAHs decreased their stomatal resistance sharply during the early hours of the first day. No significant differences were observed between the two treatments during this particular period. While following a similar overall trend as for the treated plants (Figure 4A), control plants showed significantly higher values ( $P < 0.05$ ) of stomatal resistance from dawn to mid-day ( $1.56 \pm 0.08$  &  $0.42 \pm 0.062$  s cm<sup>-1</sup> respectively). No significant difference was observed between the control and the treated plants in the second 24 hours (Fig. 4B).

Relative growth rate (RGR: mg<sub>DWT</sub> g<sup>-1</sup> d<sup>-1</sup>) of all



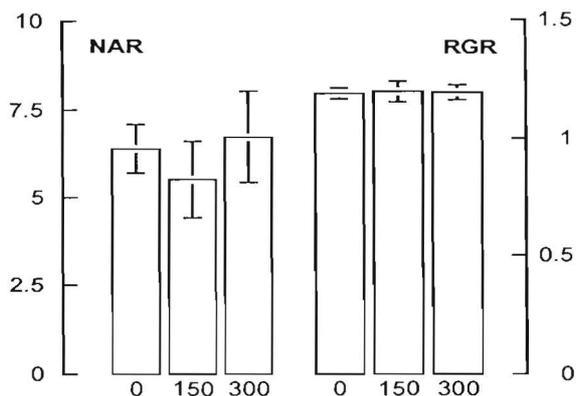
**Figure 4.** The stomatal resistance response curve of *Avicennia marina* seedlings treated with the WSF of light crude oil over 48 hours. (□); control plants, (Δ); plants treated with volumes of WSF equivalent to 150 (g PAHs plant<sup>-1</sup>, and (○); Plants treated with volumes of WSF equivalent to 300  $\mu\text{g}$  PAHs plant<sup>-1</sup> (A); measurements during day 1, (B); measurements during day 2. Values are mean  $\pm$  SE, n=10.

plants after six weeks showed no significant difference between the control and PAH treated plants either through foliar spray or soil application. Figure (5) shows the RGR of plants only sprayed with 150 and 300  $\mu\text{g}$  PAHs plant<sup>-1</sup>. Both treatments allowed plants to accumulate  $1.20 \pm 0.023$  &  $1.196 \pm 0.031$  mg<sub>DWT</sub> g<sup>-1</sup> d<sup>-1</sup> respectively, similar to the

control plants ( $1.193 \pm 0.023 \text{ mg}_{\text{DWT}} \text{ g}^{-1} \text{ d}^{-1}$ ). Net assimilation rate (NAR:  $\text{g}_{\text{DWT}} \text{ m}^{-2} \text{ d}^{-1}$ ) is a major factor controlling changes in the RGR.

Results presented in figure (5) show no significant difference in NAR between plants sprayed with 150 and 300  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$  ( $5.506 \pm 1.1$  and  $6.716 \pm 1.3 \text{ g}_{\text{DWT}} \text{ m}^{-2} \text{ d}^{-1}$ ) respectively, and the control plants ( $6.394 \pm 0.7 \text{ g}_{\text{DWT}} \text{ m}^{-2} \text{ d}^{-1}$ ).

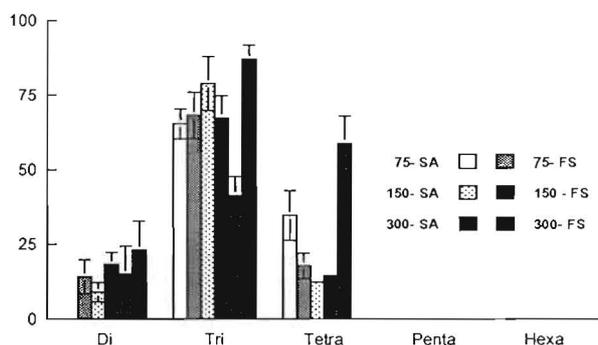
None of the five classes of PAHs was naturally



**Figure 5.** Relative growth (RGR:  $\text{mg}_{\text{DWT}} \text{ g}^{-1} \text{ d}^{-1}$ ) rate and net assimilation rate (NAR:  $\text{g}_{\text{DWT}} \text{ m}^{-2} \text{ d}^{-1}$ ) of *Avicennia marina* seedlings sprayed with volumes of the WSF of crude oil equivalent to 0 (control), 150, and 300  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$ . Values are mean  $\pm$  SE,  $n = 5$ .

detectable in the control plants; therefore, any value recorded in the treated plants is generally higher. Data shown in Figure 6, therefore, represent percentages of different classes of PAHs in the treated plants.

Naphthalene was undetectable in plants treated



**Figure 6.** Relative concentrations of different groups of PAHs (Di-, tri-, tetra-, penta-, and hexa-aromatic hydrocarbons) accumulated in *Avicennia marina* seedlings treated with different volumes of the WSF of light crude oil equivalent to 3 doses of PAHs through soil application (SA) or foliar spray (FS). The relative concentration of every aromatic group is given as a percentage of the total PAHs. Values are mean  $\pm$  SE,  $n = 5$ .

with 75  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$  through the soil. The same dose delivered to the plant through foliar spray increased the accumulated naphthalene to 14% of the total PAHs accumulated in the plants. Similarly, the spraying method increased the percentage of accumulated naphthalene in all higher doses (see, Fig. 6). Tri-aromatics represented the highest percentage of PAHs of the total. However, there was no clear pattern in the percentage accumulated of PAHs with change in the doses applied to plants (see, Fig. 6). Tetra aromatics also followed no particular order. They were higher in plants treated with 75  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$  through application. On the other hand, the higher dose (300  $\mu\text{g}$  PAHs  $\text{plant}^{-1}$ ) accumulated a higher percentage of this particular class of PAHs through foliar spray (see, Fig. 6). Penta- and hexa-aromatics were expectedly undetectable in all treatments, as they were undetectable in WSF (see, Table 1, Fig. 1 & 6).

## Discussion

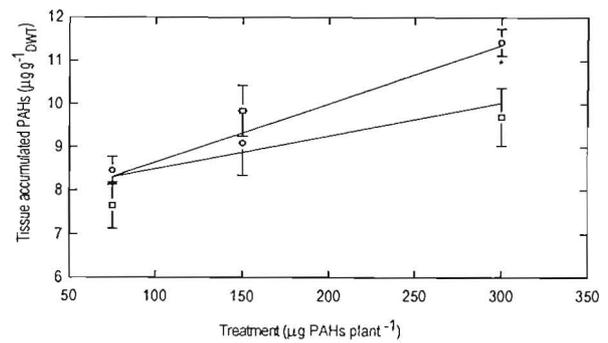
Light crudes generally have a high content of PAHs and low viscosity. These characteristics make them more toxic than heavier crudes. Under laboratory conditions, (Duke *et al.* 1998) observed a 30% mortality in *Avicennia marina* seedlings treated with weathered Arabian light crude, whereas weathered Bunker C (heavy crude) caused only 3% seedling mortality. Similar observations were reported earlier by (Getter *et al.* 1985).

Tri-aromatics are the most dominant fraction in both crude oil and aqueous extract. The differential hydrophobic nature of PAHs is evident in the relative  $K_{ow}$  values (octanol-water partition coefficient), where solubility in water increases as  $K_{ow}$  value decreases. Tri- and hexa- aromatic PAHs, for example, have  $K_{ow}$  values of 4.5 and 7.1 respectively (Karcher, 1988; Baumard *et al.* 1998; Kim *et al.* 1999). As solubility of PAHs in water decreases by increasing molecular weight, bioavailability of these compounds is expected to decrease in the aqueous fraction in the order from di to hexa-aromatic hydrocarbons (Porte and Albaigés, 1993; Djomo *et al.* 1996; Baumard *et al.* 1998). Changes in the bioavailability are clearly evident in the case of naphthalene, whose percentage to the total PAHs increased from 0.055 % in the crude to 28.98 % in the WSF.

Transpiration rate and stomatal resistance showed typical trends during the measurement period (48 hours), where transpiration rate decreased as stomatal resistance increased regardless of the treatments. However, plants sprayed with WSF showed a very weak stomatal control over transpiration rates during the first 24 hours. Stomatal control over transpiration is normally reflected by the degree of correlation between stomatal resistance and rate of transpiration (Youssef and Saenger, 1998). This irregular stomatal behavior may be explained by the possible venting of low molecular weight aromatic hydrocarbons (e.g. benzene, toluene, and xylenes) through the stomata. A similar explanation for the reduced stomatal resistance in the mangrove *Avicennia marina* was suggested earlier by (Getter *et al.*, 1985 and Youssef and Ghanem, 2002). A reduction in stomatal resistance was also observed in other non-mangrove plants fumigated with vehicle exhaust gases (Schenone *et al.* 1994; Maier-Maercker and Koch 1995; Robinson *et al.* 1998 and (Viskari *et al.* 2000). (Viskari *et al.*, 2000) showed that exhaust gases containing methyl-ethylbenzene, xylenes and toluene cause the stomata to lose their regulating capability at night.

In the present study, no significant difference in RGR was observed between the control and all PAH treatments in either way of application. The insignificant difference in RGR is further explained by the insignificant difference in the net assimilation (NAR) between treatments and the control (Ball and Pidsley, 1995; Ball *et al.* 1997). (Proffitt and Devlin, 1998) reported similar results for *Rhizophora mangle* treated with crude oil under field conditions. Furthermore, (Durako *et al.*, 1993) demonstrated that an unweathered 1 % (w/v) Kuwait crude oil WSF had no detrimental effect on the metabolic oxygen production, respiration or photosynthesis-irradiance curve characteristics of *Halophila ovalis* seagrasses.

In general, total PAHs accumulated in plant tissues increased with increasing PAH doses applied (Fig. 7).



**Figure 7.** Correlation between the application method and amounts of total PAHs accumulated in *Avicennia marina* seedlings treated with volumes of WSF equivalent to 75, 150, and 300 µg PAHs plant<sup>-1</sup>, through foliar spray (○) ( $Y=0.014x + 7.280$ ,  $r^2=0.984$ ) or soil application (□) ( $Y=0.0084x + 7.720$ ,  $r^2=0.515$ ). (\*); significantly different at  $P>0.05$ . Values are mean±SE,  $n = 5$ .

The spraying technique appears to allow more PAHs to be accumulated in plant tissues than does the soil application, particularly in the highest dose applied ( $9.7 \pm 0.67 \mu\text{g g}^{-1} \text{DWT}$  and  $11.43 \pm 0.31 \mu\text{g g}^{-1} \text{DWT}$ ,  $P>0.05$ ) (see, Fig. 7). A linear relationship was observed between the PAH doses applied to plants and the amounts accumulated (correlation coefficient,  $r^2=0.515$  for soil application and  $r^2=0.984$  for foliar spray) (see, Fig. 7). Furthermore, in the higher doses, the total PAHs accumulated in sprayed plant tissues were significantly higher than those accumulated in plants treated by soil application.

Getter *et al.* (1985) suggested that the root system is the main entry for oil hydrocarbons to the plants where it can be translocated to the shoot by the transpiration stream. The present data demonstrated that the uptake of water-soluble hydrocarbons is equally possible through the foliage, perhaps through stomatal openings or dissolution in the waxy cuticle. In addition, it appears that the entry through the foliage accumulates more hydrocarbons than does root absorption (see, Fig. 7). (Zhou *et al.*, 1998) found that soil organic matter can play an important role in determining the transformation of PAHs from the dissolved phase to the particulate phase and consequently becoming unavailable to plants. These findings may explain the difference in the physiological responses of seedlings treated with foliar and soil application. The interaction between soil organic matter and PAHs in the WSF may also account for the low value of the regression

coefficient ( $r^2=0.515$ ) for soil application compared with that of foliar spray application ( $r^2=0.984$ ). (Getter *et al.*, 1983 and Duke *et al.*, 1998) indicated that the nature of the substrate seems to have a role in the toxic effect of oil on mangrove seedlings. They found that mortality of *Avicennia marina* seedlings in response to oil treatment was more on sandy soil than in muddy substrate.

## References

- Ball, M. C. and Pidsley, S. M.** (1995) Growth response to salinity in relation to distribution of two mangrove species, *Sonneratia alba* and *S. lanceolata*, in north Australia. *Functional Ecology* **9**:77-85.
- Ball, M. C., Cochran, M. J. and Rawson, H. M.** (1997) Growth and water use of the mangrove *Rhizophora apiculata* and *R. stylosa* in response to salinity and humidity under ambient and elevated concentrations of atmospheric  $CO_2$ . *Plant Cell and Environment* **20**:1158-1166.
- Baumard, P., Budzinski, H., Garrigues, P., Sorbe, J. C., Burgeot, T. and Bellocq, J.** (1998) Concentration of PAHs (polycyclic aromatic hydrocarbons) in various marine organisms in relation to those in sediments and trophic level. *Marine Pollution Bulletin* **36**: 951-960.
- Böer, B.** (1993) Anomalous pneumatophores and adventitious roots of *Avicennia marina* (Forssk.) Vierh. mangroves two years after the 1991 Gulf War oil spill in Saudi Arabia. *Marine Pollution Bulletin* **27**: 207-211.
- Den Hartog, C.** (1984) Effect of oil pollution on seagrasses bed. Proceedings of the first international conference on the impact of oil spill in the Persian Gulf: pp 257-268.
- Djomo, J. E., Garrigues, P. and Narbonne, J. F.** (1996) Uptake and depuration of polycyclic aromatic hydrocarbons from sediment by zebrafish (*Brachydanio rerio*). *Environmental Toxicology* **15**: 1177-1181.
- Duke, N. C., Burns, K. A. and Dalhaus, O.** (1998) Effect of oils and dispersed-oils on mangrove seedlings in planthouse experiments: A preliminary assessment of results two months after oil treatment. *APPEA Journal* **1**: 631-635.
- Durako, M. J., Kenworthy, W. J., Fatemy, S. M. R., Valavi, H. and Thayer, G. W.** (1993) Assessment of the toxicity of Kuwait crude oil on the photosynthesis and respiration of seagrasses of the Northern Gulf. *Marine Pollution Bulletin* **27**: 223-227.
- Garrity, S. D., Leving, S. C. and Bruns, K. A.** (1994) The Gallate oil spill. I- Long term effects of physical structure of mangroves fringe. *Estuarine Coastal and Shelf Science* **38**: 327-348.
- Getter, C. D., Ballou, T. G. and Dahlin, J. A.** (1983) Preliminary results of laboratory testing of oil and dispersants on mangroves. Proceedings of the 1983 oil spill conference: pp 533-538.
- Getter, C. D., Ballou, T. G. and Koons, C. B.** (1985) Effects of dispersed oil on mangroves. Synthesis of a seven-year study. *Marine Pollution Bulletin* **16**: 318-324.
- Getter, C. D., Cintron, G., Dicks, B., Lewis, R. R. and Seneca, E. D.** (1984) The recovery and restoration of salt marshes and mangroves following an oil spill In: Cairns, J. and Buikema, A. L. (eds.) *Restoration of habitats impacted by oil spills*. Butterworth, London., pp 65-113.
- Grant, D. L., Clarke, P. J. and Allaway, W. G.** (1993) The response of gray mangrove *Avicennia marina* (Forssk.) Vierh. seedlings to spills of crude oil. *Journal of Experimental Marine Biology and Ecology* **171**: 273-295.
- Karcher, W.** (1988) *Spectral atlas of polycyclic aromatic compounds*. Kluwer, Dordrecht, The Netherlands.
- Kim, G. B., Maruya, K. A., Lee, R. F., Lee, J. H., Koh, C. H. and Tanabe, S.** (1999) Distribution and sources of polycyclic aromatic hydrocarbons in sediments from Kyeonggi Bay, Korea. *Marine Pollution Bulletin* **38**: 7-15.
- Klekowski, E. J., Corredor, J. E., Lowenfeld, R., Klekowski, E. H. and Morell, J. M.** (1994a) Using the mangrove to screen for mutagens in tropical marine environments *Marine Pollution Bulletin* **28**: 346-350.
- Klekowski, E. J., Corredor, J. E., Morell, J. M. and Del Castillo, C. A.** (1994b) Petroleum pollution and mutation in mangroves. *Marine Pollution Bulletin* **28**: 166-169.
- Knapp, A. H.** (1987) Effect of south Louisiana USA crude oil and dispersants on *Rhizophora* mangrove. *Marine Pollution Bulletin* **18**: 1228-1234.
- Lee, R. F. and Page, D. S.** (1997) Petroleum hydrocarbons and their effects in sub-tidal regions after major oil spills. *Marine Pollution Bulletin* **34**: 928-940.
- Maier-Maercker, U. and Koch, W.** (1995) Poor stomatal control of water balance and the abscission of green needles from a declining stand of spruce trees (*Picea abies* (L.) Karst) from the northern Alps. *Trees* **10**: 63-73.
- Page, D. S., Giliffan, E. S., Foster, J. C., Hotham, J. R. and Gonzalez, L.** (1985) Mangrove leaf tissue sodium and potassium ion concentrations as sublethal indicators of oil stress in mangrove trees. Proceedings of the 1985 oil spill conference., American Petroleum Institute, Washington D.C., pp. 391-393.
- Porte, C. and Albaigés, J.** (1993) Bioaccumulation patterns of hydrocarbons and polychlorinated biphenyls in bivalves, crustaceans and fishes. *Journal of Arch., Environmental Contamination and Toxicology* **26**: 273-281.
- Proffitt, C. E. and Devlin, D. J.** (1998) Are their cumulative effects in red mangroves from oil spills during seedling and sapling stage? *Ecological Applications* **8**: 121-127.

- Proffitt, C. E., Devlin, D. J. and Lindsey, M.** (1995) Effect of oil on mangrove seedlings grown under different environmental condition. *Marine Pollution Bulletin* **30**: 788-793.
- Ralph, P. J. and Burchett, M. D.** (1998) Impact of petrochemicals on the photosynthesis of *Halophila ovalis* using chlorophyll fluorescence. *Marine Pollution Bulletin* **36**: 429-436 .
- Robinson, M. F., Heath, J. and Mansfield, T. A.** (1998) Disturbances in stomatal behaviour caused by air pollutants. *Journal of Experimental Botany* **49**: 461-469.
- Schenone, G., Fumagalli, I., Mignanego, L., Montinaro, F. and Soldatini, G. F.** (1994) Effects of ambient air pollution in open-top chambers on bean (*Phaseolus vulgaris* L.). *New Phytologist* **126**: 309-315.
- Scherrer, P., Blasco, F. and Imbert, D.** (1989) Etude experimentale in situ de la toxicite du petrole brut et de 2 additifs envers les plantules de *Rhizophora mangle*. *Environmental Technology Letters* **10**: 323-332.
- Snedaker, S. C., Jimenez, J. A. and Brown, M. S.** (1981) Anomalous roots in *Avicennia germinans* (L.) in Florida and Costa Rica. *Bulletin of Marine Science* **31**: 467-470.
- Teas, H. J., Duerr, E. O. and Wilcox, J. R.** (1985) Effect of dispersed and non-dispersed oil on the red mangrove. *Marine Pollution Bulletin* **18**: 122-126.
- US EPA** (1996) Test methods for evaluating solid waste. Laboratory manual. Physical and Chemical methods. National Technology Information Service.
- Viskari, E. -L., Holopainen, J. K. and Karenlampi, L.** (2000) Responses of spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions - II ultrastructural changes and stomatal behavior. *Environmental Pollution* **107**: 89-98.
- Youssef, T. and Ghanem, A.** (2001) Salt Secretion and stomatal behavior in *Avicennia marina* seedlings fumigated with the volatile fraction of light Arabian crude oil. *Environmental Pollution* **116** (2): 215-223.
- Youssef, T. and Saenger, P.** (1998) Photosynthetic gas exchange and accumulation of phytotoxins in mangrove seedlings in response to soil phsico-chemical characteristics associated with waterlogging. *Tree Physiology* **18**: 317-324.
- Youssef, T. and Saenger, P.** (1998) Photosynthetic gas exchange and water use in tropical and subtropical populations of the mangrove *Aegiceras corniculatum*. *Marine and Freshwater Research* **49**: 329-334.
- Zhou, J. L., Fileman, T. W., Evans, S., Donkin, P., Llewellyn, C., Readman, J. W., Mantoura, R. F. C. and Rowland, S.J.** (1998) Fluoranthene and Pyrene in the suspended particulate matter and surface sediments of the Humber estuary, UK. *Marine Pollution Bulletin* **36**: 587-597.

Received 03/05/2000, in revised form 14/12/2001