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The Response of Two Halophytic Species to Crude Oil-Contaminated Soil in the Northern Western Region of Egypt

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ABSTRACT

A site that covers over 20 acres of coastal saline depression in the western Mediterranean coastal desert of Egypt (El-Hamra station, the main crude oil pipeline terminal in Alamein) is contaminated with petroleum. This area, prior to contamination by crude oil was dominated by different common halophytes. *Atriplex halimus* (L) and *Arthrocnemum macrostachyum* (Moric.) are now the most dominant species growing in this site. These species adapt themselves through different growth parameters, physiological and biochemical changes. Crude oil affects significantly on soil fertility and increasing pH (8.4). Importance value (IV), height and canopy diameter of the two studied species exhibited an increase in the polluted site. Organic metabolites such as fatty acids, antioxidant compounds and protein fractions in shoots of studied species which collected from the oil-contaminated and non-contaminated sites were examined. Fatty acid fractions exhibited the opposite performance especially the content of saturated C: 16 (palmitic), mono and di-unsaturated C18:1 (oleic) and C18:2 (linoleic) fatty acids as well as poly-unsaturated C18: 3 and C20: 3 (Omega -3 fatty acids). Antioxidant activity and most examined phenolic compounds were increased in *A. macrostachyum* which grow in a contaminated site. Protein fractions in *A.halimus* attained with enormous variations therefore, a Genomic Templet Stability (GTS %) was lower than in *A. macrostachyum* under contamination. The biochemical and behavioral responses to oil pollution varied with the two different species may be according to the genetic make-up of individuals, which make the two studied species useful and effective tools for phytoremediation purposes.

Key words: Adaptation; *Arthrocnemum macrostachyum*; *Atriplex halimus;* Petroleum.

INTRODUCTION

Plants are always exposed to abiotic environmental stresses. Soil contaminants are main abiotic stress factors which caused great negative effect on overall plants' growth, metabolism and hence productivity and sustainability (Wani *et al*., 2018).

Ecological pollution with petroleum oil has been recognized as a serious problem affecting the biodiversity of the ecosystems in the impacted areas (Lin *et al*., 2002; Lawer *et al*., 2013). Crude oil is a complex mixture of hydrocarbons consisting of both aliphatic and aromatic structures and toxic in nature (Al-Hawas *et al*., 2012; Shukry *et al*., 2013).

The plant community in petroleum wastes impacted site usually disappears after several months, leaving high content of weathered hydrocarbons (Lin *et al*., 2005). After some time, revegetation by the appearance of supposed oil tolerant plant species occurs (Mendelssohn *et al*., 2002), and it has been hypothesized that revegetation in these crude oil contaminated sites is a result of ecological, biochemical and physiological adjustments of these plant species to the hydrocarbons existence (Merkl *et al*., 2004).

Nowadays, these environmental problems cause dangerous damages to soil micro flora and plants. Oil physical and chemical effects caused to change metabolism and growth of plants (Treutter 2006; Yan 2018). Therefore, they affect all plant morphological and physiological parameters, photosynthetic pigments (Han *et al*., 2016) and nutrient absorption (Rosso *et al*., 2005). Since, the plants are immovable; they must have different mechanisms depending on the plant species, oil type, amount and concentration for their survival and to make them resistant to adverse environments and keep their suitability (Zhang *et al*., 2007; Besalatpour *et al*., 2008).

Notably, the halophytic species*; Atriplex halimus* and *Arthrocnemum macrostachyum* are growing in a natural eco-system at Alamin saline depression where the soil is infused with crude oil. Therefore, the main objective of this study was to determine to what range the metabolites of the selected species influence their survival in an oil-contaminated environment.

MATERIAL AND METHODS

Description of the studied sites

The study site covers over 20 acres of coastal saline depression in the western Mediterranean coastal desert of Egypt (El-Hamra station, the main crude oil pipeline terminal in Alamein) is contaminated with crude oil as a result of activities from refineries, tanker and pipeline break-ups and oilfield blowouts, (Barakat *et al*., 2001). This site is located about 120 km west of Alexandria city between 30º 80' to 31° North latitude and 29º 30' East longitudes (Figure 1). The area prior to contamination was dominated with different common halophyte species such as *Limonastrum mono-petalum, Zygophyllum album, Arthrocnemum macro-stachyum, Suaeda prunosa* and *Salsola tetranda*, (Ayyad and El-Ghareeb 1982). Now, few species were found growing in this oil-contaminated site.

Sample collection

Aerial parts (shoots) form (3-10) individuals of the two-dominant species; *Atriplex halimus* and *Arthro-cnemum macrostachyum* were selected randomly at each site (contaminated and non-contaminated) during winter 2017-2018. In the field, plant parts were carefully cleaned of the sand particles. The samples of plants and soil underneath (0-30 cm) were collected and then stored in plastic bags, brought to the laboratory shortly after collection and prepared for analysis. There were three independent replicates for plants and soil

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samples in each study site. The non-contaminated site is located about 10-20 Km east of the con- terminated site in a natural saline depression habitat.

Figure (1): Location map of the study site in the Mediterranean coastal region is presented by circle (Frithy *et al*., 2004).

Soil analysis

Three soil samples were collected from each site from the depth ranged from 0-30 cm. Soil texture was determined by the hydrometer method (Bouyoucos, 1962). Total organic matter was determined by wet oxidation according to Black (1965). Soil past extract was prepared according to Richards (1954) for estimation of pH, electrical conductivity (EC). As well as soluble cations (Ca^{++}, Mg^{++} Na^{+}, K^{+},) and anions (CO_3^{--}, HCO_3^{-}, Cl^{-},). Calcium carbonate was assayed by calcimeter method according to Black (1965). Nitrogen was determined by kjeldahl method (Jackson, 1958) and total available phosphorus according to Trough and Meyer (1929).

Vegetation analysis

Absolute density, cover, and frequency were estimated in the field using quadrat and the transect method (Mannetje, 1978). Three stands (30 X 50 m) were selected in each site and vegetation was surveyed using 25 quadrats in each stand and the cover determination was carried out along ten transects (15 m) in each stand. Relative density, relative frequency and relative cover were used to calculate the species importance value (IV) for the species existing in contaminated and non-contaminated sites. Life form spect-rum for each recorded species was identified according to Raunkiaer (1937). Plant nomenclature is according to Tackholm (1974); Boulos (2009). In addition, the height and canopy diameter of the studied species were also estimated.

Plant analysis

The plant samples from the aerial parts (shoots) of the two studied species were thoroughly washed with tap & distilled water. Thereafter, dried in an oven at 60 ºC, then ground and prepare for analysis.

Determination of the residual oil in plant and soil samples

Plant /Soil samples weight were extracted with 150 ml of high purity dichloromethane in Soxhlet for 16 h. The extract was then evaporated in a reweighed beaker to determine the weight of the residue. The residue is reported as crude oil (Short *et al*., 1996).

Fatty acids

Lipid extraction of the plant material was carried out with methanol and chloroform (Harold *et al*., 1981) and then methanolysis of the total lipids was carried out with methano-sulphuric acid (Radwan 1978). Fatty acids were analyzed using gas chromatography model HP (Hewlett Packard) 6890. The column HP-INWAX (Cross-linked polyethylene glycol) 60m, 0.25mm ID, 0.25µm film thickness. An FID detector was used at 250 ºC with an injection temperature 220 ºC the temperature program 4 ºC/min with used an initial temperature 140 ºC for 5 min. The fatty acids were identified by a comparison of the retention times with those of the standards. The double bond index (DBI) was calculated according to Campos *et al*., (2003).

Where: DBI = [(C16:1+C18:1+2×C18:2+3×C18:3)/(C16:0+C18: 0)].

Phenolic compounds

Extraction of the Plant Material

Aerial parts of both *Atriplex halimus* and *Arthrocnemum macrostachyum* from each site were dried and grinded. The dried powders (0.5 g) were extracted with methanol triplicate using sonication for 20 min each. The plant extracts were filtered and the solvents were evaporated under reduced pressure under vacuum using rotary evaporator at 50 ˚C. The dried residues were weighed and then dissolved in methanol using 50 mL volumetric flask to prepare the sample solution.

Phenolic Analysis by HPLC

High Performance Liquid Chromatography (HPLC) was performed according to Boligon and Athayde (2014). An Agilent 1260 Infinity system (Germany) equipped with a multiple wavelength ultraviolet detector (280 nm- 320 nm) performed the analysis of the phenolic compounds. The System consisted of quaternary gradient solvent pump to control the flow rate of the mobile phase (65 methanol: 35 water) and auto sampler for automatic injection, a vacuum degasser, a column oven (40˚C). Five microliters of each sample were injection onto the HPLC column using the auto sampler apparatus with a 100 µL sample loop. The separation was performed on ZORBAX Eclipse plus C18 (250× 4.6Mm id, 5 µm particle size). Data were managed using HP Chemstation software.

Antioxidant activity

The antioxidant activity was evaluated by using the stable 2, 2-diphenyll–picrylhydrazy radical (DPPH) according to a modification of the method described by Wendy Brand Williams *et al*., (1995).

Protein electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electro-phoresis (SDS-PAGE) was applied for assessing the effect of crude oil contamination on protein content and its banding pattern in the aerial parts of the studied species according to Laemmli (1970). The bands

produced by each sample were counted and the percentage of a Genomic Template Stability % (GTS %) was calculated (Cimino, 2006).

Statistical analysis

The significance of difference on the data of soil characters, height, and canopy of studied plants was calculated by Student's t- test, and values $p< 0.05$ were considered to be significant, as well as the data of hydrocarbon compounds content in the aerial parts (shoots) and roots of studied species were subjected to standard one-way analysis of variance (ANOVA) test, all to assess the significance of variations of these variables under contaminated and non-contaminated conditions using IBM SPSS software package version conditions using IBM SPSS software package version 20.0 (Kirkpatrick and Feeney, 2013).

RESULTS

Crude oil contamination affected the soil physical and chemical properties (Table 1). The content of crude oil in contaminated soil was 20.29±8.94 mg/g dry weight but not detected in non-contaminated one. Soil pH (up to 8.4), organic matter % and EC were significantly higher in contaminated than in non-contaminated soil, while total available phosphorus (AP) and nitrogen concentrations were significantly lower in the contaminated soil (Table1). Elements such as K^+, Mg^{++}, Na^+ and Cl^- were higher in contaminated soil than in non-contaminated one while Ca^{++} attained the opposite trend.

Table (1): Physical and chemical characteristics of soil and total content of petroleum hydrocarbon (TPHs) in contaminated and non-contaminated sites. Each value represents the mean ±SD (N=3).

Parameters	Site		*t*	*p*
	Contaminated	Non-Contaminated		
Texture	Sandy loam	Sandy loam	-	-
pH	8.40 ± 0.20	7.90 ± 0.10	3.873*	0.018*
EC (ds/m)	110.62 ± 3.99	73.32 ± 2.39	13.885*	<0.001*
Organic matter % (OM)	4.50±0.44	0.85±0.03	14.358*	0.005*
$CaCO_3$ (%)	36.23±2.10	41.91±2.40	3.082*	0.037*
Total nitrogen (%)	0.03±0.01	0.06±0.01	3.130*	0.035*
Available phosphorus (AP) (ppm)	5.23±0.50	8.06±0.77	5.333*	0.006*
K^+ (meq/L)	46.30±3.36	13.76±1.25	15.727*	<0.001*
Ca^{++}(meq/L)	35.63±1.37	65.58±2.51	18.143*	<0.001*
Mg^{++}(meq/L)	631.21±16.69	210.27±10.78	36.702*	<0.001*
Na^+(meq/L)	1225.41±14.83	733.19±10.57	46.818*	<0.001*
HCO_3^-(meq/L)	41.19±1.87	40.59±1.85	0.393	0.714
Cl^-(meq/L)	1404.74±15.02	857.77±8.20	55.367*	<0.001*
CO_3^{--}	ND	ND	-	-
TPHs (mg/g. dry soil)	20.29±8.94	ND	-	-

t,Student's t-test; *p*, p value for comparing between two sites; *: significant value at $p \leq 0.05$;.**, highly significant at $p \leq 0.001$; ND, Not tested.

The vegetation characteristics of the two studied sites are represented in table 2**.** Species recorded IV was different between the contaminated and uncontaminated sites. The number of species recorded in the contaminated site was more than those recorded in non-contaminated one. Notably, *A. macrostachyum* was the most dominant species in contaminated site followed by *Halocnemum strobilaceum* and *A.halimus*, while *Kochia indica*, *Phragmitis aus-trallis* and *Spergula fallax* were individualized. In non-contaminated site, *Halocnemum strobilaceum*, *A.macrostachyum* and *Limoniastrum monopetalum* were the most dominant plants.

The height and the canopy diameter of the two studied species were the highest in the contaminated site compared with non-contaminated one (Table 3). The height of *A.hilimus* increased significantly more than two times in the contaminated site. However, the canopy diameter of *A. macrostachyum* also recorded a significant increase, more than two times, in the contaminated site.

Table (2): List of the plant species recorded in the study area with their families, life form, and its importance value.

Plant species	Family	Life form	IV†	
			Con.	Non.
***Atriplex halimus* (L.)**	Chenopodiaceae	phanerophytes	40.36	9.08
***Arthrocnemum macrostachyu*(Moric)**	Chenopodiaceae	Phanerophytes	148.91	89.22
***Halocnemum strobilaceum* (Pall.)**	Chenopodiaceae	Phanerophytes	53.99	134.96
***Limoniastrum monopetalum* (L.) Boiss**	Plampagenaceae	Chamaephytes	10.97	60.29
***Zygophyllum album* (L.)**	Zygophyllaceae	Chamaephytes	20.98	6.43
***Kochia indica* (Wight)**	Chenopodiaceae	Therophyte	4.15	NR
***Phragmitis australis* (Cav.)**	Poaceae	Geophyte	21.45	NR
***Spergula fallax* (Lowe)**	Caryophyllaceae	Therophyte	7.23	NR

†**IV**, Important Value; Con, contaminated site; Non., non-contaminated site; NR: not recorded.

Table (3): The measured plant height and canopy diameter of the two studied species in the contaminated and non-contaminated sites. Each value represents the mean ±SD (N=3).

Plant species	Plant Height (cm)	Canopy diameter (cm)
***Atriplex halimus* (Con.)**	129.0±55.0	724.0±213.9
***Atriplex halimus* (Non.)**	61.0±7.0	406.7±75.1
***t* (*p*)**	**2.727*(0.049*)**	**2.415(0.052)**
***Arthrocnemum macrostachyum* (Con.)**	62.2±32.8	238.2±91.8
***Arthrocnemum macrostachyum* (Non.)**	43.3±18.6	84.1±41.3
***t* (*p*)**	**1.583 (0.131)**	**4.840**(<0.001*)**

t,Student's t-test; *p*, *p*-value for comparing between two sites.*: significantly at $p \leq 0.05$; .**, highly significant at $p \leq 0.001$. Con, contaminated site; Non, non-contaminated site.

Table (4): The content of hydrocarbons compounds (mg/g dry wt.) in shoots and roots of the two studied species in contaminated and non-contaminated sites. Each value represents the mean ±SD (N=3).

Plant species	Shoots		Roots		F	$p^{\dagger}$
	Con.	Non.	Con.	Non.		
Atriplex halimus	36.28 ±3.20 [a]	20.23 ±2.14 [b]	13.92±3.04 [b]	6.86 ±0.34 [c]	78.178*	<0.001*
Arthrocnemum macrostachyum	24.44 ±2.86 [a]	22.41 ±2.28 [a]	6.09 ±1.87 [b]	5.98 ±0.19 [b]	72.059*	<0.001*

†*: Statistically high significant at $p \leq 0.001$. Means with similar letters, in the same row, are not significantly different as results of one-way ANOVA Con., contaminated site; Non. Non-contaminated site.

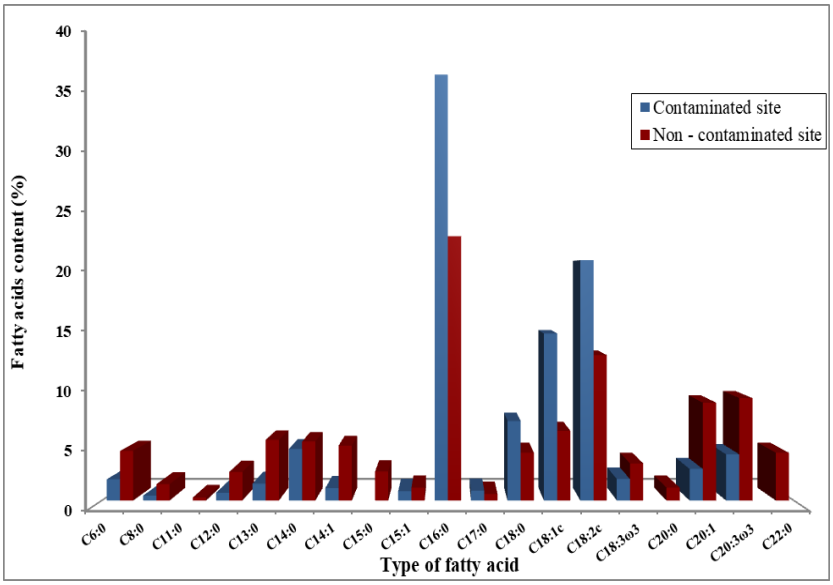

Figure (2): Fatty acid content, in percentage, of total fatty acids detected in *A. halimus* in contaminated and non-contaminated sites. The code indicates the number of double bonds. Relative content for C11, C15, C20 and C22 fatty acids in contaminated site were not detected.

Changes in the fatty acid composition of the two studied species are shown in figures 2& 3. The total content of fatty acids in *A. halimus* (Figure 2) was higher than in *A. macrostachyum* in contaminated and non-contaminated sites. The content of fatty acids in *A. halimus* in the non-contaminated site was more than in contaminated site while the opposite was recorded in *A. macrostachyum* (Table 3). Fatty acid content as percentage of total fatty acids showed that Palmitic acid (C16:0) was the major saturated fatty acid in *A. halimus* which represent about 23.1% in non-contaminated site compared to 37.2% in contaminated site. Linoleic acid (C18:2) and oleic acid (C18:1) which represented 13% and 6% in non-contaminated site and increased by 61% and 57% in contaminated one respectively. Mono-unsaturated (C20:1) and Poly-unsaturated (C20:3) and (C18:3) omega 3 fatty acids decreased due to contamination by 68% and 55% and 42.3% respectively. Four types of saturated fatty acids (C11:0, C15:0, C20:0, C22:0) in *A. halimus* disappeared in contaminated site. The ratio of total unsaturated to saturated fatty acids was calculated in order to explore

any obvious effects on the degree of unsaturation for both species in the two sites (Table 5). Slightly or mostly no difference for this ratio in both sites of each species was recorded, while double bond index (DBI) of *A. halimus* was slightly more in non-contaminated site and of *A. macrostachyum* was slightly more in contaminated one (Table 5). Most fractions of fatty acids in *A. macrostachyum* were increased in contaminated site (Figure 3). The majority of fatty acids percentage were palmitic acid (C16: 0) followed by linoleic (C18: 2) and oleic (C18: 1) and which accounting 36%, 20.2% and 7.1% of the total fatty acids respectively in non-contaminated site and decreased in contaminated one by 29.7%, 41.6% and 67.6% respectively. Polyunsaturated (Omega - 3 fatty acids) C18: 3 and C20: 3 and saturated fatty acids C22:0 increased under contamination by petroleum oil by 146% for omega 3 fatty acids and 119 % for the other. Two types of fatty acids appeared in the shoot of *A. macrostachyum* only in contaminated site (C8:0) and (C20:1).

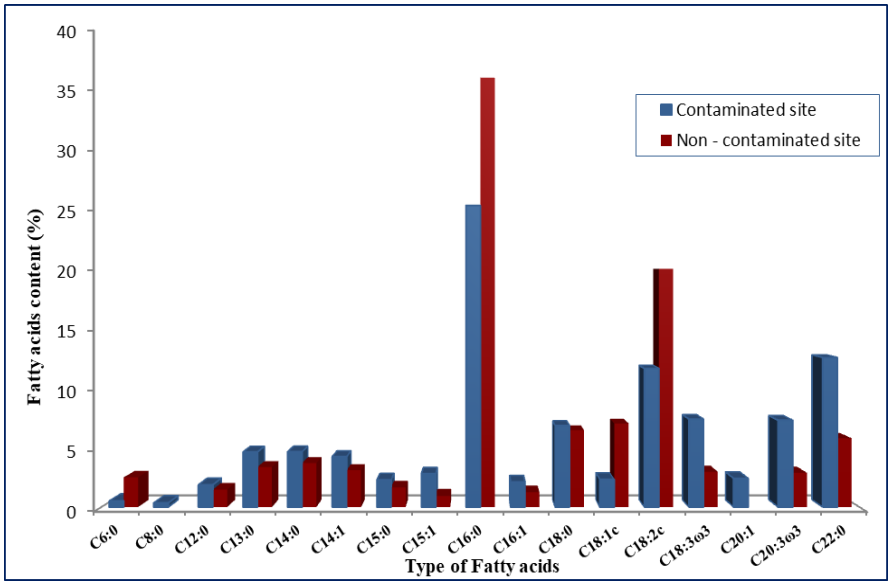

Figure (3): Fatty acid content, in percentage, of total fatty acids detected in *A. macrostachyum* in contaminated and non-contaminated sites. The code indicates the number of double bonds. Relative content for C11, C15, C20 and C22 fatty acids in contaminated site were not detected.

Table (5): Changes in fatty acid content (mg/100g of lipid) and double bond index (DBI) in the two studied species in contaminated and non-contaminated sites.

Fatty acids	*Atriplex halimus*		*Arthrocnemum macrostachyum*	
	Contaminated	Non-contaminated	Contaminated	Non-contaminated
Total saturated fatty acids	13.696	16.437	14.074	10.834
Total mono-unsaturated fatty acid	4.888	6.181	2.295	2.181
Total poly-unsaturated fatty acid	6.831	7.487	6.279	4.596
Total content of fatty acids	25.415	30.105	22.648	17.611
Ratio of total unsaturated/saturated	0.856	0.832	0.609	0.626
DBI	1.4	1.52	1.55	1.35

DBI: Double bond index.

Phenolic compound (Rutin) content showed notably change between the two studied species in contaminated and non-contaminated sites (Table 6). Rutin in *A.halimus* decreased under contamination with crude oil, while in *A. macrostachyum* was increased. Concerning with the other phenolic compounds in *A.halimus* elagic, ferulic and chlorogenic increased in contaminated site and sinapic decreased while in *A. macrostachyum* all phenolic compounds increased with contamination except chromogenic. It is to be noted that antioxidant activity(DPPH inhibition %) in both studied plants was always higher in contaminated site than in non-contaminated one (Table 6).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) as a biochemical marker technique, to evaluate pollution influence on *A. halimus* and *A. macrostachyum* collected from contaminated and non-contaminated sites (Plate1), showed different bands

of protein profile which reveals the effect of hydrocarbons on the studied plants.

Generally, *A. halimus* expressed in many protein fractions after exposure to oil pollution comparing with *A. macrostachyum*, whereas the total number of bands in *A. halimus* was 22 and 19 in uncontaminated and contaminated site respectively. The new appeared bands (95, 60, 31, 29, 27, 21, 14, 12 KDa), disappeared bands (116, 99, 72, 51, 38, 30, 28, 21.5, 20, 16, 13 KDa) and genome template stability (GTS %) was 41% in contaminated site. In *A. macrostachyum* the total number of bands was 18 and 19 in uncontaminated and contaminated site respectively. The new appeared bands (95, 72, 54, 30 KDa), disappeared bands (63, 28, 22 KDa) and (GTS %) in contaminated site was 78%.

Table (6): Phenolic compounds identified by HPLC of the methanolic extracts (mg/g.d.wt.) of the shoots of the two studied species and their antioxidant activity (DPPH inhibition %).

Constituent detected	*Atriplex halimus*		*A. macrostachyum*	
	Con.	Non.	Con.	Non.
Rutin	8.2402	12.765	10.098	2.580
Elagic	4.729	3.096	5.386	5.045
Ferulic	0.703	0.380	1.891	0.676
Sinapic	0.585	0.948	1.492	0.806
Chlorognic	0.141	0.094	0.163	0.197
DPPH Inhibition (%)	92.05	63.58	91.18	85.55

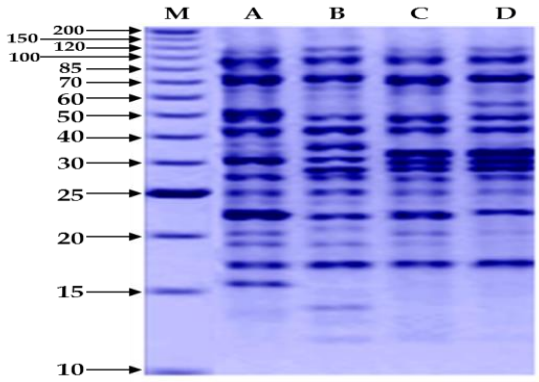

Plate (1): SDS-PAGE of Proteins isolated from leaves of *A. halimus* and *A. macrostachyum* in contaminated and uncontaminated sites; Lane A-*A. halimus* in non-contaminated site; Lane B-*A. halimus* in contaminated site; Lane C-*A. macrostachyum* in non- contaminated site; Lane D-*A. macrostachyum* in contaminated site; M - Protein marker.

DISCUSSION

In natural environments, plants respond to a combination of a biotic stresses like total petroleum hydrocarbons by different ways including morphological, physiological and even molecular response. Total petroleum hydrocarbons (TPHs) represent a group of serious pollutants of the environment with great impact on living organisms.

Regarding the physicochemical properties of contaminated soil, the crude oil contamination could potentially alkalinize the soils (8.4) and adversely affect soil fertility and physical properties, and hence cause deterioration to the soil (Wang *et al*., 2013). This may be explain why $CaCO_3$, TN, PO_4, and Ca were lower in contaminated site compared to non- contaminated one which may be used by soil micro flora and explain the imbalance in C/N ratio (Akunne, 2007).

Concerning the vegetation characters, the species height as well as crown cover were lower in contaminated than non - contaminated site, this may be due to the petroleum contamination reduce cell activity or even plant mortality (Lichtenthaler, 1996). Despite the ability of vegetation for adaptation and certain stress tolerance mechanisms, vegetation usually responds to sudden short-term or long-term stressors with reduced cell activity and reduced plant growth or even plant mortality. The stress factors vary in their intensity and duration which can cause damage to plant vegetation (Lichtenthaler, 1996). On the other hand, (Noomen *et al*., 2012) found that vegetation cover and species diversity are affected by hydrocarbon wastes. In the present study the IV, height and canopy diameter of the two studied species which indicate their biomass were more in contaminated site than in non-contaminated one. These results may be due to species tolerant to the determined content of hydrocarbons (20.29 mg/g d.wt.±8.94) in the contaminated soil. (Robson *et al*., 2004) showed that hydrocarbon pollution caused a decrease in vegetation cover and species richness, while some species were found to be tolerant. IV and richness are different between contaminated and non-contaminated site may be due to the variation between species abilities to tolerate crude oil (Racine, 1994), soil disturbance (Wali, 1999), low fertility (Wilson and Tilman 1991), difficult in the consumption of water and mineral salts from the soil, and also interruptions a number of metabolic processes (Rusin *et al*., 2015).

Organic molecules from crude oil can penetrate living plants through their roots and leaves from where the hydrocarbon compounds can be transported into the plant vascular system and intercellular spaces leading to cell and tissue damage, this effect depends on the type and amount of oil and the plant species (Baker, 1970).

The regulation of fatty acid biosynthesis may be an important means of controlling the membrane fatty acid unsaturation, contributing to the plant adaptation (Chaffai *et al*., 2009). Fatty acids are crucial components of cellular membranes, suberin, and cutin waxes that provide structural barriers to the environment (Beisson *et al*., 2007). The fatty acids of the two studied species were examined in response to crude oil pollution exhibit the opposite performance to each other. Whereas, in *A. halimus* most saturated fatty acid fractions and unsaturated fatty acids decreased especially (C20:3 and C18: 3) omega 3. This may be a result of free radical mediated oxidation of triple bonds. These highly reactive free radicals have strong affinities for the cell membrane (Hussein and Norman 2002). Also, the content of saturated (palmitic) and unsaturated (oleic and linolenic acid) increased under oil contamination. Carla and Maria (2018) stated that palmitic acid (C16:0) is building blocks for numerous regulations of membrane fluidity. Vereshchagin *et al*. (1985); Shayakh *et al*., (1990) stated that, linolenic and

palmitic acids inherent to plant tissues under cold stress, these acids are believed to allow the cell adapt itself under adverse conditions at the expense of a rise in membrane fluidity and that is why it's absolute and relative level in the tissue rises. This unsaturated fatty acid (C18:3) may be maintaining membrane permeability (Badea and Basu 2009). In *A. macrostachyum* exhibit the opposite trend, increase of most saturated fatty acid fractions and unsaturated fatty acids especially (C20:3 and C18: 3) omega three. Also, the content of palmitic, unsaturated (oleic, linolenic and myristoleic acid) decreased under oil contamination. Anttonen *et al*., (1995) reported an increase in saturated fatty acids such as myristic, with a significant reduction in unsaturated fatty acids such as linolenic acid in ozone-exposed Aleppo pine (P. *halepensis* Mill.). This suggestion may be supported by the lower ratios of unsaturated to saturated fatty acids in *A. macrostachyum* growing at the contaminated site of the present study. The increase in DBI was probably due to a higher C18: 3 percentages and to a decrease in the more saturated fatty acids in the newly synthesized lipids. It may also depend on compositional changes resulting from lipid turnover (Campos *et al*., 2003).

Plant organisms being devoid of motility and immune system; have elaborated alternative defines strategies, involving the enormous variety of secondary metabolites (phenolic compounds) as tools to overcome stress constraints, adapt to the changing environment and survive (Vasconsuelo and Boland 2007). Secondary metabolites are compounds that are not necessary for an organism to live, but play a role in the interaction of the organism with its environment. These compounds are often involved in plants protection against biotic or abiotic stresses. Secondary metabolites are from different metabolites families that can be highly inducible in response to stresses (Pagare *et al*., 2015).

In the present study secondary metabolites mostly increased in contaminated site especially in *A. macrostachyum.* Ramakrishna and Ravishankar (2011) stated that the accumulation of such metabolites often occurs in plants subjected to stresses including various indicator molecules.

Most environmental stresses are affecting the production of active oxygen species in plants, causing oxidative stress (Hendry 1994 and Bartosz 1997). Also, there is growing evidence that in plants subjected to environmental stress the balance between the production of activated oxygen species and the activity of antioxidant is upset, which often results in oxidative damage (Smirnoff 1993; Karpinski *et al*., 1997). DPPH inhibition which represent the antioxidant activity of the two studied plants showed great values in contaminated site compared with uncontaminated one this may be due to the increase in most studied phenolic compounds under oil pollution (Elagic, Ferulic and chlorogenic in *A.halimus*) but (rutin, Ferulic and sinapic in *A. macrostachyum*). These are potential antioxidants and may work as ROS-scavenging compounds (Olga *et al*., 2003). Moreover, it has been shown recently that phenolic compounds can be involved in the hydrogen peroxide scavenging cascade in plant cells (Takahama and Oniki, 1997).

Proteins are compounds of fundamental importance for all functions in the cell (Dose, 1980). It is well known that alteration of gene expression is always involved in formulating plants for survival under stress. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions (Vierstra, 1993; Hieng *et al*., 2004). The adaptation of protein biosynthesis and its structure under ecological stress factors such as chemical pollutants are of particular interest, frequently act together and trigger adaptive and protective mechanisms (Vinocur and Altman, 2005). One of the most important mechanisms involved in the cell protection against stress is the induction of de novo synthesis of a set of proteins (Kermode, 1997), these set of proteins were newly synthesized under drought stress conditions. In the present study, protein fractions in *A. halimus* attained with enormous variations (appearance and disappearance bands) therefore, GTS% was lower than in *A. macrostachyum* under oil contamination. The synthesis of new protein fractions with different intensity revealed that the important role of protein in the resistance to stress imposed by crude oil. Tardieu (2013) stated that the physiological and behavioural responses to environmental condition depend on the genetic make-up of individuals which varied for expression and activity levels also plant species, oil kind, amount and concentration, exposure times and environmental condition (Zhang *et al*., 2007; Besalatpoor, 2008).

The studied species are growing abundantly and tolerant to crude oil pollution which may make them a useful tool for phytoremediation purposes. These results agree with (Malgorzata *et al*., 2017) who found that Lotus *corniculatus* L. and *Oenothera biennis* L. can be used as a phytoremediator species of polluted soil with petroleum hydrocarbons. The present study will show more promise for detailed studies and applications.

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استجابة نوعان من النباتات الملحية للتربة الملوثة بالزيت الخام في المنطقة الشمالية الغربية لمصر

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الملخص العربـــــي

يوجد خط انابيب رئيسي للنفط الخام في منطقه العلمين بالساحل الشمالي الغربي لمصر (محطة الحمرا للبترول) الواقعه في المنخفض الملحي علي بعد حوالي 120 كم غرب اسكندريه. ونتيجة الانشطة من المصافي البترولية ومخلفات الزيت الناتجة عن تصريف صيانة صهاريج التخزين العائمة والتخلص من هذه البقايا خلال فترة سنوات عديدة في 20 فدانا من الأرض في هذه المنطقة ادى الى تلوث التربة بالزيت الخام . كانت هذه المنطقة قبل التلوث يسود فيها النباتات الملحية المعروفة بوجودها في هذا الموطن والان بعد تلوث التربة بالبترول نجد غياب لمعظم هذه النباتات وان نبات القطف ونبات بصاق هما النوعان الاكثر شيوعا ونمو في هذا الموقع. وتهدف هذه الدراسة الى معرفة كيف اثرت التربة الملوثة بالنفط الخام على ايض بعض المواد الاولية (المواد الدهنية والبروتينية) و الثانوية (بعض المركبات الفينولية ومضادات الاكسدة) وكذلك قياسات النمو لهذه النباتات الصحراوية التي تكيفت ونمت في هذا الموقع. وجود النفط الخام في التربة في هذه المنطقة اثر بشكل معنوي على خصوبتها وزاد من درجة حموضتها مما اثر على قيمة الاهمية وارتفاع وقطر نباتي الدراسة حيث تبين ان هذه القياسات زادت في الاماكن الملوثة بزيت البترول. كما اظهرت النتائج ان هذان النباتان لهم قدرة على امتصاص المركبات الهيدروكربونية من التربة وكان لنبات القطف قدرة اعلى من البصاق على امتصاص هذه المركبات. وفي الدراسة تم تقدير الاحماض الدهنية و المركبات المضادة للاكسدة و كذلك فصل حزم البروتين في المجموع الخضري لهذان النباتان في الاماكن الملوثة بالبترول وغير الملوثة بالبترول كمرجع. اوضحت النتائج ان الاحماض الدهنية اظهرت اداء معاكس في النباتين المدروسين خاصة في محتوى الاحماض الدهنية المشبعة وخاصة (البالماتيك) و الاحماض الدهنية الاحادية والثنائية غير المشبعة (الاوليك و اللينوليك) وكذلك بعض من الاحماض الدهنية العديدة غير المشبعة (اوميجا 3). واظهرت النتائج زيادة في نشاط مضادات الاكسدة ومعظم المركبات الفينولية المدروسة في نبات البصاق النامي في الموقع الملوث بالنفط و الحزم البروتينية في نبات القطف اظهرت اختلافات هائلة في المكان الملوث وبالتالى استقرار الهيكل الجيني فيه كان اقل منه في نبات البصاق. واستنتج من الدراسة ان هناك اختلاف بين نباتي الدراسة في الاستجابات الفسيولوجية والبيوكيماوية والسلوكية تحت تاثير التلوث بالبترول وتمكن كل نبات منهم بهذه الاستجابات من النمو والبقاء في هذا الموقع الملوث بالبترول و قد يعزى ذلك الى التكوين الوراثي لهذه الانواع النباتية والتي مكنتها من النمو في البيئة الملوثة مما قد يجعل هذان النوعان من النباتات اداة مفيدة وفعالة في اغراض المعالجه النباتيه لهذة الملوثات.