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Research Article

Adaptation potential of two common halophytes to salinity stress in the Salt Marshes of lake Burullus in Egypt

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Abstract

This study aims to investigate the physiology of adaptation mechanisms to increase salinity from wet to dry season when the salts are accumulated on the soil surface. *Arthrocnemum macrostachyum* (Moric.) K. Koch. and *Halocnemum strobilaceum* (Pallas) M. B are the common native plants to the salt marshes of Burullus Lake at the deltaic Mediterranean coast of Egypt. The two halophytic plants survive and grow in the prevailing high concentrations of salts. Plants and soil samples were collected from six locations depending on changes of their soil EC values. During wet season which have high rain and low temperature, the two halophytes accumulated high content of the primary metabolites carbohydrates, amino acids, proline and proteins. On the opposite at the dry season, due to increased temperature salinity increased greatly, even salt crust appears on the soil surface. The two halophytes in face to this high salinity accumulated secondary metabolites (alkaloids, phenolic compounds and polyamines) during the dry than the wet season. The high content of anthocyanins especially in *A. macrostachyum* showed their utilization in the two plants as a main source for pigments and antioxidants.

Key words: Alkaloids, Amino Acids, Burullus lake, Salt marshes, Halophytes, Phenolic Compounds, Proline, Protein and Polyamines.

1. INTRODUCTION

Burullus Lake is a shallow brackish lake extending for 47 km along the deltaic Mediterranean coast of Egypt and its width about (5-11 km)¹. The source of lake water is drainage water through human activities, rainfall and Mediterranean Sea water through Bughaz El-Burullus. The halophytes represent the vegetation area of Burullus lake salt marshes. Halophytes are over 2000 species in the world, species of natural flora, form a group of ecologically biologically, physiologically and biochemically specialized plants capable of functioning normally and reproducing on saline soils. Halocnemum strobilaceum and Arthrocnemum macrostachyum are succulent perennial halophytes characterized by articular stems with carnose segments, reduced and stems-united leaves, and occur in highly saline environments². Halophytes are naturally salt-tolerant plants that may be potentially useful for economical applications (oilseed, forage, production of metabolites)³. Halophytes are plants adapted to live in saline environment, sea water, a

salt-water marsh or a salt-desert⁴. Salt tolerance depends, to a great extent, on the cellular compartmentalization of toxic ions; tolerant plants have the ability to accumulate Na⁺ and Cl⁻ in the vacuole, so that the cytoplasm is maintained at substantially lower ion concentrations, thus avoiding the inhibition of metabolic processes 56 . The maintenance of osmotic balance requires the synthesis and accumulation in the cytoplasm of compatible solutes (osmolvtes), which are not inhibitory to the metabolism even at high concentrations. These organic compounds are carbohydrates and amino acids and derivatives such as proline, glycine betaine⁷⁸⁹¹⁰ High proline content can be considered beneficial to stressed plants. Significant correlation between enhanced tolerance and proline accumulation in plants under saline condition has been reported¹¹. Environmental stresses (salinity, drought) may trigger oxidative stress in plants, generating the formation of reactive oxygen species (ROS), leading to cellular damage, metabolic

disorders, and senescence processes ^{12,13}. Some antioxidant compounds are extracted from easy sources, such as agricultural and horticultural crops, or medicinal plants. Among them, halophytes are naturally salt-tolerant plants that may be potentially useful for economical applications as new sources of natural antioxidants in dietary food¹⁴. A close correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in numerous crops, such as pea, cotton, rice^{15,16,17}. Plant organs produce a wide variety of secondary metabolites (such as alkaloids, phenolic compounds and polyamines) which may play numerous biological activities¹⁸. Salinity stress affects the total alkaloids and phenolic compounds content in the plant. Salinity induces disturbance of the metabolic processes leading to an increase in secondary metabolites^{19,20,21}. These metabolites have an osmoregulatory role and considered as an adaptation mechanism to the imposed stress²². Phenolic compounds exhibit a wide spectrum of biological activities such as anti-allergic, anti-artherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects^{23,24}. Polyamines, putrescine, spermidine, spermine, and cadaverine are low molecular weight organic polycations displaying a high biological activity. polyamines, both in the free and soluble conjugated forms, were suggested to have an important protective role against stresses such as cold, wilting, pollution, osmotic, salt, drought, heat, chilling and oxidation stresses^{25,26}.

2. MATERIALS AND METHODS

2.1 Plant material

Arthrocnemum macrostachyum (Moric.) K. Koch. and Halocnemum strobilaceum (pallas) M. B. represent the main vegetation of salt marshes of Burullus Lake of Egypt. The two plant species were collected during 2009 in winter (February), spring (May), summer (August) and autumn (November) from the following locations along the costal international road and they are at;

1- El hanfe village, 2- Mostroa village, 3- Godaa village,4- El maksaba village, 5- Emad village 6- Borg el Burullus village

The locations were arrangement in accordance with the increase in their salinity. The two plants samples were thoroughly washed with tap water followed by distilled water and then dried in an oven at 60°C for constant weight. The dried materials were powdered using an electric mill.

2.2 Soil analysis

Soil samples were taken within and away from the root systems of the two plants at depth (0-30cm) and their 1-5 soil solution electrical conductivity (EC)

was measured using an electrical conductivity meter 27 .

3. PLANT ANALYSIS

3.1- Estimation of carbohydrates

Dry shoot (0.1g) was extracted for 24 hours with a borate buffer. The borate buffer supernatants were used for estimating soluble carbohydrates. The residue was used for estimating polysaccharides quantitatively using the modified method²⁸.

3.2 Estimation of total free amino acids

Amino acid was determined from 0.1 g of the dried plant sample and the amino acid content was expressed as mg per g dry mass. Proline was determined in dry shoot samples and the proline content was expressed as mg proline per g dry mass^{30,31}.

3.4 Quantitative estimation of total soluble proteins

The total soluble proteins content was estimated quantitatively in the borate buffer extract ³². The protein content was calculated as mg/g dry mass using a prepared calibration curve by Bovine Serum Albumin protein.

3.5 Estimation of total alkaloids and phenolic compounds

Total alkaloids were measured quantitatively and expressed as mg/g dry mass of the plant samples³³. Total phenolic content was estimated quantitatively and expressed as mg/g dry mass of the plant sample³⁴.

3.7 Determination of polyamines

Putrescine, spermidine and spermine, were extracted and the content (ppm) of each polyamine of *A. macrostachyum* and *H. strobilaceum* was determined in four locations during summer season only³⁵.

3.8 Statistical analysis

The results were statistically analyzed using the two ways analysis of variance (ANOVA) to determine the F test, LSD at 0.05 level and the degree of significance for the obtained variations by different locations, different seasons and their interactions and the analysis was carried out by **SPSS statistical package**³⁶.

4. RESULTS

4.1 Soil analysis

The characteristics of soil samples under and away from the two studied plant species (*Arthrocnemum macrostachyum* and *Halocnemum strobilaceum*) varied at the six locations 1, 2, 3, 4, 5 and 6 in the salt marshes of Burullus Lake and during the four seasons (Table 1). The soil electrical conductivity (EC) show that the maximum mean (8.9 mS cm⁻¹) was in the summer season while the minimum one (3.9 mS cm⁻¹) was in the spring season. The mean values of EC in the soil away from the plants were higher than under the two studied plant species in most location and during winter and spring seasons. Also, the soil EC under *Halocnemum macrostachyum* at all season especially at winter when soil EC under the later plant was double under the former plant. The highest EC values were recorded in location 6 during all seasons in the soil under and away from plants while the lowest one was in location 1.

4.2 Carbohydrates content

A- The direct reducing value (DRV)

Table 2 showed that the DRV of *A. macrostachyum* and *H. strobilaceum* shoot were remarkably varied in response to the effect of salinity of the different locations during all seasons. *A. macrostachyum* the highest value was 80 mg g⁻¹ d.m. at location 6 in the winter season, while the lowest value was 26 mg g⁻¹ d.m. at location 2 in summer season. *H. strobilaceum* acquired greater DRV sugars during most seasons compared to *A. macrostachyum*, the highest value was 99 mg g⁻¹ d.m. at location 5 in the spring season, while the lowest value was 25 mg g⁻¹ d.m. at location 3 in winter season.

The highest value of sucrose content in *A*. *macrostachyum* was 38.6 mg g⁻¹ d.m. at location 6 in the spring season, while the lowest value was 4.80 mg g⁻¹ d.m. at location 3 in winter season in *A*. *macrostachyum*. In *H. strobilaceum* the highest value was 40.0mg g⁻¹ d.m. at location 3 in the spring season, while the lowest value was 3.6 mg g⁻¹ d.m. at location 2 in winter season.

The highest value of starch content in *A*. *macrostachyum* was 2.71 mg g⁻¹ d.m. at location 3 in the spring season, while the lowest value was 1.12 mg g⁻¹ d.m. at location 6 in summer season in *A*. *macrostachyum*. Starch content was remarkable greater in *H. strobilaceum* than in *A. macrostachyum*. The highest starch content in *H. strobilaceum* was 31.1 mg g⁻¹ d.m. at location 1 in the spring season, while the lowest one was 11.3 mg g⁻¹ d.m. at location 6 in summer season.

The order of variation of the three carbohydrate components was DRV > sucrose > starch showing low storage of carbohydrate in the two plants although there is a marked content of starch in *H. strobilaceum* (Fig. 1). The highest contents of the three sugar components and in the two halophytic plants were at the active growing spring season when edaphic factors were suitable.

4.3 Amino acids and protein content

The results represented in table 3 showed that the content of both amino acids and proteins followed the same trend of variation by seasons. Amino acids content was greater than that of protein in both plants and during all seasons of study. Proline was greatly accumulated in the two plants at the wet season (winter). It is also high in them during the dry season. The highest value of proline content was 7.10 mg g⁻¹ d.m. at location 6 in the winter season, while the lowest value was 0.1 mg g⁻¹ d.m. at location 3 at summer season in *A. macrostachyum*. In *H. strobilaceum* the highest value was 6.0 mg g⁻¹ d.m. at location 6 in the winter season, while the lowest value was 0.13 mg g⁻¹ d.m. at location 2 in summer season.

The highest value of amino acids content was 146 mg g^{-1} d.m. at location 5 in the spring season, while the lowest value was 21 mg g^{-1} d.m. at location 2 during summer season in *A. macrostachyum*. In *H. strobilaceum* the highest value was 174 mg g^{-1} d.m. at the same location (5) and also in the spring season, while the lowest value was 50 mg g^{-1} d.m. at location 1 during summer season.

The highest value of total protein content in *A.* macrostachyum was 7.2 mg g⁻¹ d.m. at location 3 in the winter season, while the lowest value was 1.45 mg g⁻¹ d.m. at location 1 during spring season. Similarly, in *H. strobilaceum* highest value was 6.22 mg g⁻¹ d.m. at location 6 in the spring season, while the lowest value was 1.19 mg g⁻¹ d.m. at location 2 during winter season.

4.4 Alkaloids and phenolic compounds content

The mean of alkaloids content was greater than phenolic content in the two plants during all seasons except winter (Table 4). Also the trends of variation of both alkaloids content and phenolic compounds content were similar in the different seasons and in both plants with a maximum accumulation during the hot dry season (summer). The highest value of total alkaloids was 93.0 mg g⁻¹ d.m. at location 6 during summer season, while the lowest value was 11.7 mg g⁻¹ d.m. at location 5 during winter season in *A. macrostachyum*. In *H. strobilaceum* the highest value was 99.4 mg g⁻¹ d.m. at location 6 during summer season, while the lowest value was 6.80 mg g⁻¹ d.m. at location 6 during summer season.

The highest value of total phenolic was 37.8 μ g g⁻¹ d.m. during summer season at location 5, while the lowest value was 3.7 μ g g⁻¹ d.m. at location 6 during spring season in *A. macrostachyum*. Also, in *H. strobilaceum* the highest value was 42.0 μ g g⁻¹ d.m. during summer season at location 6, while the lowest

value was 6.40 $\mu g \ g^{\text{-1}}$ d.m. at location 3 during spring season.

4.5 Polyamines content

Table 5 showed that the maximum contents of putrescine, spermidine and spermine were 7.90, 10.98 and 4.24 ppm respectively in location 1 in *A. macrostachyum.* In *H. strobilaceum* the highest amounts of spermidine and spermine were 1.0 and 5.8 ppm respectively in location 6, while the highest amount of putrescine was 14.9 ppm in location 5.

5. DISCUSSION

The soil EC of the studied locations in the salt marshes of Burullus Lake was significantly increased in the summer season (dry season) due to the shortage of rain water which is required for leaching salts and decreases the soil salinity. On the opposite in winter and spring seasons (wet seasons) the rainfall led to a marked decrease of salinity. These results are in compatible with 37,38,39,40 . The EC in the near soil was lower than the soil EC under A. macrostachyum. and H. strobilaceum plants because the two halophytic plants had the ability to accumulate salts in their tissues and their died parts release salts under the plants as also indicated by 41,42,43 . It was observed that some halophytes act as phytoremediating plants by eliminating the heavy metals from the soil⁴⁴. Also, phytoremediation could be differed by the halophytic plants as the soil EC under H. strobilaceum was greater than that under A. macrostachyum at all season especially at winter when soil EC under the later plant was double that under the former plant.

The reducing sugars of shoot were generally increased with high soil EC values in all seasons especially in plants of locations 5 and 6, and shoots accumulate reducing sugars than roots. There is a reduction in the soluble sugars during the dry season that might be a result of the general inhibition in the photosynthetic pigments (chlorophyll a and b) and due to its conversion into secondary metabolites, alkaloids and phenolic compounds.^{45,46} Results that the starch indicated contents of Α. macrostachyum and H. strobilaceum were low during the summer and autumn seasons (dry season) especially in location-5 and 6. Statistical analysis revealed that the starch content was negatively correlated with soil-EC values, proline, soluble carbohydrates, amino acids, alkaloids and phenolic compounds for both plants during most seasons⁴⁸. This is due to the synthesis of starch was enhanced by the low salinity, but high salinity inhibited it. Also, starch can be converted to soluble sugars which play an important role in adjusting the osmotic potential of the cytoplasm; as concluded in some halophytic

plants^{52,53,54,44,55}. The decrease in starch content and the increased level of sucrose of both plants with high salinity pointed out a shift in the balance of sucrosestarch metabolism. Under saline conditions, the accumulation of sucrose in plants was usually considered to be the result of inhibition in sucrose oxidation in relation to shoot growth or as osmotic adjustment ^{47,48}. On the other hand, *H. strobilaceum* attained high starch content compared to that of A. macrostachyum. This may be regarded as another protectant substance against dry conditions and high temperature, though H. strobilaceum withstand more sever conditions than A. macrostachyum (field observations). Hydrophyllic compounds could replace water at the surface of proteins, complex and membranes, thus acting as osmo-protectants^{49,50}. In addition to its function as an osmoregulator, proline may protect enzyme-proteins from ion inhibitory effect⁵¹. It also stabilizes cellular structures and functions as source of carbon and nitrogen for metabolism as well as a regulator of cytosolic pH that could protect plant tissues against osmotic stres^{52,53}. So proline accumulation in plants was stimulated by increasing EC value at different locations particularly at location-5 and 6 of most seasons for both plants. Proline protects the plants by scavenging reactive oxygen species (hydroxyl radicals and superoxide radical), so the proline play the role of antioxidant compounds in direct quenching of free radical reactions during winter and spring seasons ⁵⁴. Earlier studies revealed that the possible stimulation of total phenolics via the pentose phosphate pathway may be through steps involved in proline synthesis ⁵⁵. Results indicated high values of proline and low values of phenolic compounds during winter season in A. macrostachyum and during spring season in H. strobilaceum the reverse was found in both plants during summer season. Data showed a remarkably high level of amino acids in location 5 and 6 in both plant species during most different seasons this can be attributed to the stimulation of synthesis and accumulation of amino acids with a rising in salinity for osmoregulation. These results are in agreement with Youssef (2009)⁵⁶. It was observed that alkaloids usually synthesized from amino acids in particular, lysine, tyrosine, and tryptophan where there was (De Luca, 1993)^{57.} A clear relationship was between the free amino acids content and secondary metabolites which may be increased during dry season especially in summer season instead of amino acids (primary metabolites)48. So that the results indicated that the level of free amino acids was low in both plant species during summer and autumn (dry seasons) compared to the wet seasons (winter and spring). These results are in agreement with ⁸. An accumulation of free amino acids with a decrease in protein of *Brassica napus* was detected during water stress ^{59,60}. This can be ascribed to enhanced protease activity, as an adaptive mechanism to stress tolerance ⁶¹. Plants of *H. strobilaceum* accumulate high amount of free amino acids than *A. macrostachyum* during all seasons, on the other hand *A. macrostachyum* accumulate high amount of soluble protein content than *H. strobilaceum*, which showed different mechanisms between them in osmoregulation. The reduction in the soluble protein content under elevated saline conditions may be due to:

1- Stimulating protein hydrolysis by salinity ⁶² and

2- Shortage of nitrogen supply due to exposure to NaCl in which Cl- inhibits NO3- uptake ⁶³

3- Inhibition of some regulatory enzymes for the process of protein synthesis such as nitrate reductase ⁶⁴

4- Reduced RNA content which is needed for protein synthesis ⁶⁵. The decrease in protein content during summer (dry) season may be due to conversion of many primary metabolites (carbohydrates and amino acids) into secondary metabolites (alkaloids and phenolics). Gómez-Galera *et al.* (2007)⁶⁶ stated that the phenylpropanoid pathway originates from phenylalanine and it is perhaps the most important biosynthetic pathway derived from any amino acid. The pathway is responsible for the synthesis of thousands secondary metabolites including lignins, salicylates, coumarins, hydroxycinnamic amides, flavonoid phytoalexins, pigments, UV light protectants, and antioxidants. These metabolites are often specific to a particular plant species.

The present results showed that the total alkaloids content of both plant species was generally increased by increasing salt concentration at different locations particularly location-5 and 6 during most seasons. However the alkaloids content recorded the highest values in summer and autumn (dry) seasons in comparison with winter and spring (wet) seasons for both plants, where plants used this mechanism (secondary metabolites) at more stressed time. These results are in accordance with ⁶⁷. The alkaloids have an osmoregulatory role and their increase was considered as an adaptation to the imposed salinity stress⁶⁹ reported that the increase in the alkaloids content as influenced by NaCl is a combination of an osmotic effect and a specific ion effect. The increase of alkaloids in response to salinity may be due to its role in the plant protection against the salt stress effects⁶⁸.

Many authors reported that accumulation of the phenolic compounds in plants by NaCl stress leading to consider that secondary metabolites may play a role in the adaptation of halophytic species to this constraint ⁷⁰. The present results indicated that both plants possessed high level of total phenolic compounds in the summer and autumn seasons (dry season) compared to the winter and spring seasons. The total phenolic compounds remarkably increased with salinity at different locations during different seasons, where locations-5 and 6 recorded the highest value during most seasons. The total phenolic compounds increased in the summer season on account of carbohydrates content which decreased in the same season. The phenolic compounds play an important physiological and ecological role, being involved in resistance to different types of stress ⁷¹⁷². Plants have different adaptive mechanisms to reduce oxidative damage resulting from salt stress through a cascade of antioxidants which stopping the propagation of oxidative chain reactions. In this case, polyphenolic compounds such as phenolic acids, flavonoids, proanthocyanidins and anthocyanins play an important role in scavenging free radicals ⁷⁰.

The presence of large amounts of polyamines in the studied plant species was confirmed by ⁷³ who reported that cultivars accumulating large amounts of free polyamines, exhibit a higher tolerance to osmotic stress than other cultivars. Over expression of the arginine decarboxylase (ADC2) gene in Arabidopsis results in increased putrescine level and drought tolerance ^{74,75}. Polyamine biosynthesis involves two alternative pathways starting from L-arginine ⁷⁰. Putrescine was considered as the first compound produced from the two pathways, so it acts as precursors for spermidine and spermine. They observed the following interesting fact: under high salinity, A. macrostachyum accumulated spermidine in spite of low concentrations of its precursors (putrescine), the content of putrescine increased only during low salinity; thereafter, the content of polyamines dropped sharply several assumptions could explain these results. It is naturally to suppose that, during primary response to salinity, A. macrostachyum synthesized actively putrescine which was later used as substrate for spermidine and spermine synthesis ⁵⁴ or due to shortage in primary metabolites (amino acids) in summer season. In general, the polyamine contents of A. macrostachyum decreased at high levels of salinity 76,77. The total polyamines content was low at locations 5 and 6 which are distinguished by high salinity. The decrease in polyamines may be due to their conversion into secondary metabolites such as nicotine or tropane alkaloids in Solanaceae or in their conjugation with hydroxycinnamic acids or with proteins, hemicellulose or lignin⁷⁸.

		Soil electrical conductivity					
Seasons	Locations	Under	Under	Soil away from			
		A. macrostachyum	H. strobilaceum	plants			
	1	0.2±0.1	0.7±0.1	0.30±0.2			
	2	0.5±0.1	1.1±0.2	2.00±0.1			
****	3	1.2±0.1	5.5±0.2	4.40±0.1			
Winter	4	2.1±0.2	5.4±0.1	6.90±0.1			
	5	3.4±0.2	7.0±0.1	11.2±0.1			
	6	5.4±0.2	8.9±0.4	13.5±0.1			
	Mean	2.1	4.7	6.4			
	1	0.4±0.06	1.7±0.1	0.5±0.06			
	2	1.6±0.06	2.3±0.1	1.70±0.1			
G . •	3	2.2±0.06	3.4±0.1	3.4±0.06			
Spring	4	3.2±0.06	3.8±0.1	3.80±0.1			
	5	5.0±0.06	4.6±0.1	5.80±0.1			
	6	9.6±0.10	6.8±0.1	11.0±0.2			
	Mean	3.7	3.8	4.4			
	1	1.97±0.1	4.70±0.5	2.00±0.3			
	2	3.43±0.3	4.90±0.4	3.90±0.3			
0	3	6.70±0.1	6.10±0.4	6.97±0.1			
Summer	4	6.90±0.1	6.20±0.4	7.13±0.1			
	5	8.10±0.2	16.6±0.4	14.1±0.3			
	6	17.9±0.4	21.0±0.1	21.4±0.3			
	Mean	7.5	9.9	8.7			
	1	1.00±0.3	2.00±0.3	1.60±0.5			
Autumn	2	2.80±0.3	3.00±0.4	3.10±0.4			
	3	5.00±0.4	3.60±0.3	5.70±0.3			
	4	5.60±0.4	5.00±0.3	6.60±0.4			
	5	7.10±0.5	8.90±0.3	8.60±0.5			
	6	13.8±0.5	16.0±0.4	17.0±0.7			
	Mean	5.9	6.4	6.4			

Table 1

Electrical conductivity (EC) of soil under and away from *Arthrocnemum macrostachyum* and *Halocnemum strobilaceum* at different locations during different seasons in salt marshes of Burullus Lake.

 Table 1. continue (Statistical analysis)

Table 1. continue (Statistical analysis)									
Factors	Р	LSD	Р	LSD	Р	LSD	Р	LSD	
Season	0.01	3.0	0.01	3.1	0.01	2.7	0.01	2.8	
Location	0.01	2.0	0.01	5.2	0.01	2.0	0.01	5.0	
Plants	0.01	-	0.01	-	1.00	-	1.00	-	
Season* Location	0.01	-	0.01	-	0.01	-	0.01	-	
Season* plants	0.01	-	0.01	-	1.00	-	1.00	-	
Location* plants	0.01	-	0.01	-	1.00	-	1.00	-	
Season* Location* plants	0.01	-	0.01	-	1.00	-	1.00	-	

at uniterent locations during uniterent seasons in sait marsnes of bur unus Lake.											
	A. macrosta			1	H. strobilaceum						
location	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn			
	DRV										
1	36	46	38	30	45	90	65	50			
2	30	50	26	27	30	76	45	57			
3	35	37	27	39	25	50	55	45			
4	38	52	32	40	37	60	54	55			
5	42	61	43	50	32	99	62	70			
6	80	58	54	49	38	92	67	67			
Mean	43.5	50.7	36.7	39.2	34.5	77.8	58.0	57.3			
	Sucrose										
1	8.8	33.8	12	23	5	15.4	11.2	30			
2	10	13.8	12	24.6	3.6	36	15	26.8			
3	4.8	25.8	10	28	5.4	40	20	16.6			
4	13	28.8	14	26.4	6.2	24	22.8	20			
5	15	30.6	16.8	28.8	8.4	32.8	30	32.8			
6	16	38.6	19	26.2	9	36	18	20			
Mean	11.3	28.6	14.0	26.2	6.3	30.7	19.5	24.4			
	Starch										
1	1.41	2.49	1.31	1.57	18	31.1	14.1	17.2			
2	1.42	2.65	1.32	1.68	17	30	13.5	18.3			
3	1.47	2.71	1.28	1.95	15.6	30.3	12.9	15.4			
4	1.47	2.6	1.28	1.55	15.1	30.5	12.6	13			
5	1.4	2.33	1.23	1.56	15.3	27.8	11.7	14.8			
6	1.28	2.54	1.12	1.52	15.6	27.5	11.3	13.5			
Mean	1.4	2.6	1.3	1.6	16.1	29.5	12.7	15.4			

 Table.2

 Seasonal changes of DRV, sucrose and starch content (mg g⁻¹ d.m.) of A. macrostachyum and H. strobilaceum at different locations during different seasons in salt marshes of Burullus Lake.

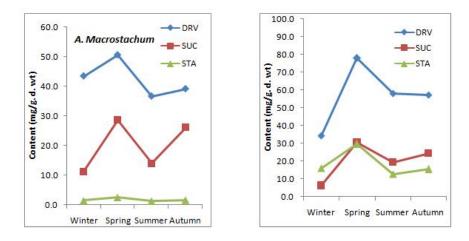


Fig. 1 The content of DRV, sucrose and starch during the different seasons of study in each *H. strobilaceum* and *A. macrostachyum*.

<i>strobuaceum</i> at unterent locations in sait marsnes of Burunus lake.												
	A. macrosta					H. strobilaceum						
location	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn				
	Proline											
1	2.7	1.1	0.6	0.9	2.4	0.84	0.31	1.4				
2	2.4	0.9	0.3	1.6	2.3	0.94	0.13	2.4				
3	2.3	1.6	0.1	1.9	3.5	0.35	0.24	2.2				
4	3.8	0.9	0.2	1.2	3.3	0.58	0.25	1.9				
5	4.6	1.8	1.6	1.9	5.5	2.6	0.45	2.6				
6	7.1	1.3	0.5	2.3	6.0	1.19	0.44	2				
Mean	3.8	1.3	0.6	1.6	3.8	1.1	0.3	2.1				
	Amino acid	s										
1	78	92	51	66	105	118	50	81				
2	92	108	21	52	113	125	66	98				
3	80	89	41	70	94	106	60	94				
4	105	117	32	77	119	119	76	100				
5	115	146	91	97	148	174	81	102				
6	119	138	71	106	160	165	78	95				
Mean	98.2	115.0	51.2	78.0	123.2	134.5	68.5	95.0				
	Protein											
1	6	1.45	5.5	4.4	1.8	3.51	2.93	3.2				
2	4.9	3.04	2	4.1	1.19	2.57	2.32	3.5				
3	7.2	3.44	2.6	4.7	2.3	1.7	2.6	3.1				
4	5.6	3.65	3.8	6	2.5	3.18	2.9	3.4				
5	4.7	6.51	4.8	5.9	2.4	5.32	4.2	3.8				
6	5.6	5.23	4.7	5	2.6	6.22	3.3	4.2				
Mean	5.7	3.9	3.9	5.0	2.1	3.8	3.0	3.5				

Table 3 Seasonal changes of proline, amino acids and protein content (mg g⁻¹ d.m.) of *A. macrostachyum* and *H. strobilaceum* at different locations in salt marshes of Burullus lake.

 Table.4

 Seasonal changes of alkaloids and phenolic content (µg g⁻¹ d.m.) of A. macrostachyum and H. strobilaceum at different locations in salt marshes of Burullus Lake.

	A. macrostac	hyum			H. strobilaceum			
location	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
	Alkaloids							
1	17.3	17	66	40	14.3	12.3	51.7	36.7
2	13.3	13.7	47.7	44.3	10.7	13	57.3	33.3
3	14.7	12.7	49.2	41.7	8.8	11.3	44	43.3
4	15.3	14.7	51.7	51.7	11.7	14.7	53.3	41.7
5	11.7	17	75	75.3	8.3	16	83.3	68.3
6	13.3	18.7	93	81.7	6.8	18.3	99.4	64
Mean	14.3	15.6	63.8	55.8	10.1	14.3	64.8	47.9
	Phenolics							
1	13.5	5.3	24.9	22.4	11.4	8.5	18.5	27
2	20.1	7.6	20.4	18	10.4	6.7	29.8	28.6
3	20.2	8.6	30.1	27	10.7	6.4	30.5	23.6
4	19	7.7	33.9	24.6	11.6	7	31.8	24.4
5	13	5.5	37.8	30.6	13	9.6	36.5	40
6	10.4	3.7	31.9	34.3	14	13.1	42	37.1
Mean	16.0	6.4	29.8	26.2	11.9	8.6	31.5	30.1

Table. 5
Changes of polyamines content (ppm) of A. macrostachyum and H. strobilaceum in four locations during
summer season only

	Polyamines (ppm)										
		A. macrostachyum		H. strobilaceum							
	Putrescine	Spermidine	Spermine	Putrescine	Spermidine	Spermine					
1	7.9	11	4.24	6.1	0.2	2.1					
3	4.1	4.5	3.1	10.5	0.6	3.3					
5	1.2	1.2	0.9	14.9	0.8	4.4					
6	2.7	1.43	3.01	12.8	1	5.8					
Mean	3.98	4.53	2.81	11.08	0.65	3.90					

On the other hand, putrescine in *H. strobilaceum* shoot recorded high amount in comparison with spermidine and spermine, and this may be due to the retro conversion from spermine and spermidine to spermidine and putrescine, respectively by plant polyamine oxidases ⁷⁹. In general the amount of polyamines of *H. strobilaceum* increased with salinity and recorded high levels at locations 5 and 6. These results were compatible with many researchers $\frac{80,81,82}{2}$.

6. CONCLUSION

Our conclusion included that these halophyte plants requires more studies for the medicinal properties due to its high production of secondary metabolites (alkaloids and phenolic compounds).

Use of both halophyte species for heavy metal remediation is of particular interest since these plants are naturally present in soil characterized by excess of toxic ions, mainly sodium and chloride.

7. REFERENCES

- Shaltout KH, Khalil MT. Lake Burullus (Burullus protected area). Publication of national Biodiversity unit. No. 13, EEAA, Cairo 2005.
- Castroviejo S. Flora Ibérica. Plantas vasculares de la Península Ibérica Islas Baleares. Vol. II. Madrid: Real Jardín Botánico, CSIC, 1990; 524-534.
- 3. Single RS, Glenn EP, Squires V. Growth performance of lambs fed mixed diets containing halophyte ingredients, Anim. Feed Sci. Technol., 1996; 63(1-4):137-148.
- Flowers TJ, Colmer TD. Salinity tolerance in halophytes. New Phytol., 2008; 179:945-963.
- Flowers TJ, Hajibagher MA, Clipson NJM. Halophytes. Qur. Rev. Biol., 1986; 61: 313-337.

- 6. Serrano R, Gaxiola R. Microbial models and salt stress tolerance in plants. Critical Reviews in Plant Sci., 1994; 13(2):121-138.
- Mosallam HA, Abd EI-Maksoud KA. Ecological studies of some desert plant growing in different saline microhabitats in salhyia area, Desert Inst. Bull., Egypt., 1996; 46:9-28.
- Crowe JH, Hoekstra FA, Crowe CM. Anhydrobiosis. Annu. Rev. Plant Physiol., 1992 54:579–599.
- 9. Elhaak MA. Effect of nitrogen deficiency and proline initiator on response of the coastal dune species *Euphorbia paralias* to salinity. JKAU: Met. Env. Arid Land Agric. Sci., 1999; 10:31-44
- 10. Mansour MMF. Nitrogen containing compounds and adaptation of plants to salinity stress. Biol. Plant, 2000; 43(4):491-500.
- 11. Ashraf M, Foolad MR "Roles of glycine betaine and proline in improving plant abiotic stress resistance". Env. and Exp. Botany, 2007; 59(2):206-216.
- Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Sankari S, Panneerselvam R. Paclobutrazol enhances photosynthesis and ajmalicine production in Catharanthus roseus, Process Biochem., 2007; 42(11):1566-1570.
- 13. Ksouri R, Megdiche W, Koyro HW, Abdelly C. Responses of halophytes to environmental stresses with special emphasis to salinity. Adv. Bot. Res., 2010; 53:117-145.
- Meot-Duros L, Le Floch G, Magné C. Radical scavenging, antioxidant and antimicrobial activities of halophitic species. J. of Ethnopharmacology, 2008; 116(2):258-262.

- Kartal N, Sokmen M, Tepe B, Daferera D, Polissiou M, Sokmen A. Investigation of the antioxidant properties of Ferula orientalis L. with suitable extraction procedure. Food Chemistry, 2007; 100(2):584-589.
- Gossett DR, Millhollon EP, Lucas MC. Antioxidant responses to NaCl stress in salttolerant and salt-sensitive cultivars of cotton, Crop Sci., 1994; 34:706–714.
- Navarro JM, Flores P. Changes in the contents of antioxidant compounds in pepper fruits at ripening stages, as affected by salinity. In: Food Chemistry, 2006; 96(1): 66–73.
- Flowers TJ, Troke PF, Yeo AR. The mechanism of salt tolerance in halophytes. Annul. Rev. Plant Physiol., 1977; 28:89-121.
- Dhingra HR, Varghese TM. Effect of growth regulators on the *in vitro* germination and tube growth of maize (*Zea mays* L.) pollen from plants raised under sodium chloride salinity. New Phytol., 1985; 100:563-569.
- Elhaak MA, Wegmann K. Ecophysiological studies on *Euphorbia paralias* under soil salinity and sea water spray treatments. J. Arid Environ., 1997; 35(3): 459-471.
- 21. Flowers TJ, Troke PF, Yeo AR. The mechanism of salt tolerance in halophytes. Annul. Rev. Plant Physiol., 1977; 89-121.
- 22. Hellebust J A. Osmoregulation. Annu. Rev. Plant Physiol., 1976; 27: 485-505.
- Orlova YV, Myasoedov NA, Kirichenko EB, Balnokin YV. Contributions of Inorganic Ions, Soluble Carbohydrates, and Multiatomic Alcohols to Water Homeostasis in *Artemisia lerchiana* and *A. pauciflora*. ISSN 1021-4437, Russian J. of Plant Physiol., 2009; 56(2): 200–210.
- Siddhuraju P. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. Lebensmittel-Wissenschaft und-Technologie, 2007; 40(6): 982–990.
- 25. Paschalidis KA, Moschou PN, Aziz A, Toumi I, Roubelakis-Angelakis KA. Polyamines in grapevine: an update, second ed., Grapevine Molecular Physiology & Biotech., Springer Sci., 2009; 2007–2028.
- 26. Shevyakova NI, Shorina MV, Rakitin VY, Kuznetsov VIV. Stress-Dependent Accumulation of Spermidine and Spermine in the Halophyte *Mesembryanthemum crystallinum* under Salinity Conditions.

ISSN 1021-4437, Russian J. of Plant Physiol., 2006;53(6): 739–745.

- Jackson ML. Soil chemical analysis constable and co. Ltd. London., 1962; P. 496.
- 28. Nelson N. A photometric adaptation of somagi method for the determination of glucose. J. Bio1. Chem., 1944; 153:275.
- 29. Naguib M1. Colorimetric estimation of plant polysaccharides. Zucker, 1963; 16:15-118.
- Misra PS, Mertz ET, Glover DV. Studies on corn proteins. VIII. Free amino acid content of *opaque-2* double mutants. cereal chem., 1975; 52:844.
- 31. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant and soil, 1973; 39:205-207.
- 32. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal-Biochem, 1976; 72:248-254.
- Harbone JB. Phytochemical methods, a guide to modern techniques of plant analysis. Chapman and Hall. London, 1973; 185-186.
- 34. Jindal KK, Singh RN. Phenolic content in male and female Carica papaya: A possible physiological marker for sex identification of vegetable seedlings. Physiol. Plant., 1975; 33:104-107.
- 35. Maijala RL, Eerola SH. Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. Meat Sci., 1993; 35:387-395.
- 36. Bishop ON. "Statistics in Biology". Longman, Penguin, London, 1983; 56-63.
- Gupta RK, Abrol IP. Salinity build-up and changes in the rice-wheat system of the Indo-Gangetic Plains. Exp. Agri., 2000; 36:273-284.
- Herczeg AL, Dogramaci SS, Leany FWJ. Origin of dissolved salts in a large, semi-arid groundwater system: Murray Basin, Australia. Marine and Freshwater Research, 2001; 52:41–52.
- 39. Cai X, McKinney DC, Rosegrant MW. Sustainability analysis for irrigation water management in the Aral Sea region. Agricultural Systems, 2003; 76:1043–1066.
- 40. Sarraf M. Assessing the Costs of Environmental Degradation in the Middle East and North Africa Countries. Environmental Strategy Notes (No. 9), Environment Department, World Bank: Washington, DC, 2004.

- Redondo-Gómez S, Mateos-Naranjo E, Figueroa ME, Davy AJ. Salt stimulation of growth and photosynthesis in an extreme halophyte, *Arthrocnemum macrostachyum*. Plant Biology, 2009;1435-8603.
- 42. Hamada EAM. Contribution on the physiology of adjustment of halophytes to saline environments. Ph. D. Thesis, Tanta. Univ., Egypt 1984.
- El-Shourbagy MN, Ahmed AM, Osman ME, Hamada EM. Adjustment of different halophytes to Mediterranean salt marshes of north Egypt. Phyton (Austria), 1984; 24:101-112.
- Eid MA, Eisa SS. The use of some halophytic plants to reduce Zn, Cu and Ni in soil. ISSN 1991-8178 Australian Journal of Basic and Applied Sciences, 2010; 4(7):1590-1596.
- 45. Omar MS, Yousif DP, Al-Jibouri AJM, Al-Rawi M S, Hameed MK. Effect of gamma rays and sodium chloride on growth and cellular constituents of sunflower (*Helianthus annus* L.) callus cultures. J. Isl. Acad. Sci., 1993; 6:(1) 69-72.
- Herrmann KM, Weaver LM. The shikimate pathway. Annu. Rev. Plant Physiol. Plant Mol. Biol., 1999; 50:473-503.
- Greenway H, Munns R. Mechanism of salt tolerance in non-halophytes. Ann.Rev. Plant Physiol., 1980; 31:149-190.
- 48. Gao Z, Sagi M, Lips SH. Carbohydrate metabolism in leaves and assimilate partitioning in fruits of tomato (*Lycopersicon esculentum* L.) as affected by salinity. Plant Science, 1998; 135(2):149– 159.
- Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol., 2000; 51:493-499.
- 50. Lambers H. Dry land salinity: A key environmental issue in southern Australia. Plant Soil, 2003; 257.
- 51. Solomon A, Beer Y, Waisel GP, Jones P, Paleg LG. Effect of NaCl in the carboxylating activity of Rubisco from *Tamarix jordanis* in the presence and the absence of proline-related compatible solutes. Physiol. Plant., 1994; 9: 198–204.
- 52. Shetty K. Role of proline-linked phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. Process Biochem., 2004; 39(7):789-803.

- 53. Kishor PBK, Sangam S, Amrutha RN, Sri Laxmi P, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr. Sci., 2005; 88(3):424-438.
- 54. Shevyakova NI, Bakulina EA, Kuznetsov VIV. Proline Antioxidant Role in the Common Ice plant Subjected to Salinity and Paraquat Treatment inducing Oxidative Stress. Russian J. Plant Physiol., 2009; 56(5):663-669.
- 55. Perry PL, Shetty K. A model for improvement of proline during Pseudomonas-mediated stimulation of rosmarinic acid levels in oregano shoot clones. Food Biotechnol., 1999; 13:137-54.
- Youssef AM. Salt Tolerance Mechanisms in Some Halophytes from Saudi Arabia and Egypt. Research J. Agri. Biol. Sci., 2009; 5(3):191-206.
- De Luca V. Enzymology of indole alkaloid biosynthesis. In: Lea PJ (ed) Methods in plant biochemistry. Enzymes of secondary metabolism. Academic, London, 1993; 345– 368.
- Alex R, Angell LM, Rocky N, Nicholas AP. Indirect and direct effects of salinity on the quantity and quality of total amino acids in *Ulva ohnoi* (Chlorophyta) Journal of Phycology, 2015; 51(3):536–545,
- 59. Good AC, Zaplachinski ST. The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. Physiol. Plant, 1994; 90:9-14.
- 60. Yong Y, Xueyong L, Yuanli J, Zuoxiang X, Qingguo X, Na Z, Bichao S. Root Growth, Free Amino Acids, and Carbohydrates of Tall Fescue in Response to Soil Salinity HortScience, 2015; 50(4):609-614
- 61. Ramanjulu S, Sudhakar C. Drought tolerance is partly related to amino acid accumulation and ammonia assimilation: a comparative study in two mulberry genotypes differing in drought sensitivity. J. Plant Physiol., 1997; 150(3):345-350.
- 62. Uprety DC, Sarin MN. Physiological studies on salt tolerance in *Pisum sativum* (L):II. Mechanism of salt action during germination. Acta. Agron. Sci. Hung., 1975; 24:188-191.

- 63. Dean-Drummond CE, Glass ADM. Studies of nitrate influx into barely roots by the use of ³⁴ClO³ as a tracer for nitrate-Iinteractions with chloride and other ions. Can. J. Bot., 1982; 60:2147-2153.
- 64. Plant Z. Nitrate reductase activity of wheat seedling during exposure to and recovery from stress and salinity. Physiol. Plant., 1974; 30:212-217.
- Bajaj Y. Effects of gamma irradiated sugars on the growth and development of plant tissues. Z Pflanzenphysiol, 1971; 3(5):418-426.
- 66. Gómez-Galera S, Pelacho AM, Gené A, Capell T, Christou P. The genetic manipulation of medicinal and aromatic plants. Plant Cell Rep., 2007; 26(10):1689-1715.
- Elhaak MA, Migahid MM. Comparison between effect of soil moisture and salinity stress on the total alkaloids in *Euphorbia paralias*. J. Union Arab Biol, Cario. Ctyogenetics, Ecology and Taxonomy, 1999; 9:59-70.
- Elhaak MA, Wegmann K. Ecophysiological studies on *Euphorbia paralias* under soil salinity and sea water spray treatments. J. Arid Environ, 1997; 35(3): 459-471.
- 69. Winkel-shirley B. Flavonoids biosynthesis: a colorful model for genetic, biochemistry, cell biology, and biotechnology. Plant Physiol., 2001 126(2):485-493.
- Kusano T, Yamaguchi K, Berberich T, Takahashi Y. Advances in polyamine research in 2007, J. Plant Res., 2007; 120(3):345-350.
- 71. Delalonde M, Barret Y, Coumans MP. Development of phenolic compounds in maize anthers (*Zea mays*) during cold pretreatment prior to endrogensis. J. Plant Physiol., 1996; 149:612-616.
- 72. Ayaz FA, Kadioglu A, Turgut R. Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthe setosa* (Rosc.) Eichler. Can J. Plant Sci., 2000; 80:373-378.
- 73. Velarde-Buendi´a AM, Shabala S, Cultivarikrova M, Dobrovinskaya O, Pottosin I. Salt-sensitive and salt-tolerant barley varieties differ in the extent of potentiation of the ROS-induced K? efflux by polyamines. Plant Physiol Biochem, 2012; 61:18-23.

- 74. Alcázar R, Planas J, Saxena T, Zarza X, Bortolotti C, Cuevas J, Bitrián M, Tiburcio AF, Altabella T. Putrescine accumulation confers drought tolerance in transgenic Arabidopsis plants over-expressing the homologous Arginine decarboxylase 2 gene. Plant Physiol. Biochem, 2010;48(7):547– 552.
- 75. Pottosin I, Velarde-Buendı'a AM, Bose J, Zepeda-Jazo I, Shabala S, Dobrovinskaya O. Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: implications for plant adaptive responses. J Exp Bot, 2014; 65:1271-1283.
- 76. Janicka-Russak M, Kabała K, Młodzi ska E, Kłobus G. The role of polyamines in the regulation of the plasma membrane and the tonoplast proton pumps under salt stress. Journal of Plant Physiology, 2010; 167(4):261-269.
- 77. Bueno M, Lendínez ML, Aparicio C, Cordovilla MP. Effect of salinity on polyamines and ethylene in Atriplex prostrata and Plantago coronopu. BIOLOGIA PLANTARUM, 2015; 59 (3):596-600.
- 78. Creuss JA, Eucuentra A, Gavalda EG, Barcelo J. Binding of polyamines to different macromolecules in plants. In: Galston AW, Tiburcio AFN (Eds.), Polyamines as Modulators of Plant Development. Ediciones Peninsular Madrid 1991; pp.30–34.
- 79. Urano K, Yoshiba Y, Nanjo T, Igarashi Y, Seki M, Sekiguchi F, Yamaguchi-Shinozaki K, Shinozaki K. Characterization of Arabidopsis genes involved in biosynthesis of polyamines in a biotic stress responses and developmental stages. Plant, Cell Environ., 2003; 26:1917-1926.
- Bassard J, Ullmann P, Bernier F, Werck-Reichhart D. Phenolamides: Bridging polyamines to the phenolic metabolism. Phytochemistry, 2010; 71(6):1808–1824.
- 81. Groppa MD, Benavides MP. Polyamines and a biotic stress: recent advances. Amino Acids, 2008; 34(1):35-45.
- Alcázar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T. Involvement of polyamines in plant response to a biotic stress. Biotechnol. Lett, 2006; 28(23):1867–1876.