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Evaluation of Ionic Osmotica in Succulent and Non-succulent Xero-halophytes Inhabiting Hot Oases

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ABSTRACT

This research was carried out at Kharga and Dakhla oases, in the western Egyptian desert. The species investigated include basically those of different ecological affiliations and different life forms, to have comparative indications on the ionic means of adjustment. During winter and summer, the water-soluble ions for both of soil and plants were analyzed. The total osmotic water potential and the share of ionic radicals of plants were also calculated. The data revealed that halophytic species were able to maintain osmotic adjustment due to the accumulation of ions, depending on seasonal and species variations, and the possession of ionic osmotic potential that related to chlorides, sodium and potassium. The seasonality or location has the dominant effect on Na^+, K^+, Cl^- and SO_4^{-2} concentrations in halophytes, *Suaeda monoica* and *Cressa cretica*, and also affected by the interaction between both factors (S x L) in the case of *Zygophyllum coccenium*. The ionic osmotic potential of Na^+/K^+ and Cl^-/SO_4^{-2} ratios for salt tolerance in studying halophytic species were also discussed.

Keywords: Ionic, osmotica, succulents, halophytes, hot areas.

INTRODUCTION

The influence of climate on plants becomes of primary importance for those areas most affected by aridity. In arid and semiarid regions, the aridity depends on the amount of water available and on the temperature which is more relevant to plant life. However, the capabilities of plants to utilize the available water under ionic or non-ionic stresses reflect the magnitude of their adaptability to thrive the severe conditions in their habitats. The ionic stress due to arise of NaCl in the rooting medium has adverse effects on plant growth and development. Mostly, these effects are osmotic stress, ion toxicity, antagonism and imbalance of ion specificity. Furthermore, the ionic and osmotic effects disturb aerobic metabolism and induce the accumulation of reactive oxygen species (ROS) beyond the plant's capacity for cellular oxidant detoxification, which in turn adversely affects cellular structures and metabolism (Chaves, *et al*., 2009).

With the accumulation of NaCl in the leaves of some halophytes and use it as an osmoticum, the down-regulation of Na^+ uptake transporters will be toxic (Katschnig *et al*., 2015). Accordingly, halophytes will adjust osmotically to soil salinity by accumulating ions mainly sodium and chloride. Therefore, cation transporters and channels exhibited to be involved in Na^+ and K^+ homeostasis in plants (Suzuki *et al*., 2016b). This means that, the low cytosolic K^+ concentrations, which participates in many physiological functions in plants, leads to severe metabolism impairment and ended with growth inhibition. Therefore, a high K^+/Na^+ ratio can be manipulated by different mechanisms that function to: (1) reduce Na^+ influx into root cells; (2) compartmentalize Na^+ into vacuoles; (3) increase Na^+ efflux from root cells (Tester and Davenport, 2003; Pardo and Rubio, 2011).

Salt tolerance of many Xero-halophytes in their habitats has mechanisms to survive with salt stress such as osmotic tolerance, ion exclusion, and tissue tolerance (Roy *et al*, 2014; Munns and Tester2008). These criteria can be evaluated by the osmoionic regulation of water potential by the investigated species. Investigating Na^+ and K^+ homeostasis in plants, grown under saline condition, may increase the understanding of salt stress tolerance mechanisms. This can be declared by estimation of the osmotic water potential of Na^+, Cl^-, K^+ and SO_4^{-2} in plants inhabit hot Egyptian oases. The data obtained, concern this study, for both soil and studied plant are evaluated by statistical analyses.

MATERIAL AND METHOD

This work was carried out on wild halophytes inhabiting saline areas with soil texture ranged between sandy to silty soil for Kharga (Locations 1-6) and Dakhla (Locations 7-9) oases and adjacent lands in the western desert of Egypt (Map,1). Soils and plants were sampled twice: in mid-winter conditions and in harsh summer climate to cover the seasonal changes. The measured parameters in response to changes in climatic conditions were tabulated (Table, 1). Both soil and plant samples were collected from some sites (stands) which represent its distribution at different habitats in both oases.

Soil samples and collection technique

Soil samples were collected from the rooting zone of the investigated plants, by digging down around the root zone, from different selected locations. The studied locations were site 1 (Port-Said); site 2 (Ganah); site 3 (Bolaque); site 4 (Sanaa); site 5 (Gazayer); site 6 (South Max); site 7 (Teneida); site 8 (Asmant) and site 9 (Qalamoon). For each site, three replicates of the soil samples (chosen at random) and sampled from surface

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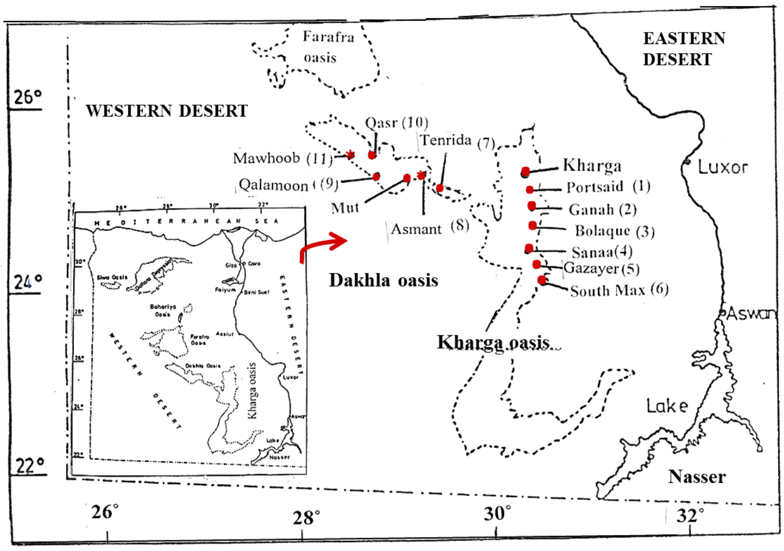

Map (1): The study area and sampling locations.

(0-5 cm), and sub-surface soil (at depth 20-25 cm), then transferred in a clean plastic container to the laboratory. The percentage of water content in the soil samples was calculated according to the following equation:

$$\%\ of\ water\ content = \frac{Wet\ soil - Dry\ soil}{Dry\ soil} \text{ X } 100$$

Preparation of soil extracts

Water extract of each air-dried and sieved soil sample was prepared at the ratio of 1:5 (soil/dist. H_2O). The soil extracts were kept in the deep freeze until the time of chemical analyses. These analyses include: total soluble salts (TSS); anions, sulphates and chloride, and cations such as sodium and potassium ions. TSS was assessed by evaporation of a soil water extract according to the following the equation:

$$\%\ of\ TSS = \frac{Wt.of\ total\ soluble\ salts}{Volume\ of\ soil\ extract}\ X\ dilution\ X\ 100$$

For anions, sulphate and chloride were determined according to Black *et al*., (1965) and Jackson (1958), respectively. Meanwhile, sodium and potassium were determined according to the method of Williams and Twine (1960).

Collection of plant samples

Plant specimens of four native species were collected from their natural habitats in the sites studied, when encountered. The Plant specimens were identified according to Täckholm (1974) and Boulos (1999, 2000, 2002, 2005). The sampled plant species identified as follow: *Suaeda monoica* Forssk., *Salsola imbricate* Forssk., (F: Chenopodiaceae), *Cressa cretica* L.(F: Convolvulaceae) and *Zygophyllum coccineum* L. (F: Zygophyllaceae). The collected plant materials, branches bearing leaves, were immediately transferred to tightly close plastic containers, which then were transferred to the laboratory for further investigation. Samples of leaves were washed with distilled water and thoroughly dried on filter paper. For each species, four samples were chosen at random, then oven-dried at 70°C for 24 hrs. and reweighed to calculate their water content as follows:

$$\%\ of\ water\ content = \frac{Fresh\ Plant\ Wt - Plant\ Dry\ Wt}{Fresh\ Plant\ Wt.} \text{ X } 100$$

The relative water content (RWC) of leaves was expressed as a percentage and evaluated according to Weatherly and Barrs (1962) as follow:

$$RWC = \frac{Fresh\ Wt\ of\ leaf\ cuts - Dry\ Wt\ of\ leaf\ cuts}{Saturation\ Wt\ of\ leaf\ cuts - Dry\ Wt\ of\ leaf\ cuts}$$

Preparation of plant extracts for analysis

After determination of the dry weight, 0.1 gram of finely powdered oven-dried material of each plant sample was transferred to a clean test tube. Ten ml of bi-distilled water was added and heated to 80-90°C in a water bath for a one hour, stirred at intervals and then filtration was done by using filter paper according to El-Sharkawi and Michel (1977). Plant extracts were kept in vials in deep freeze ready for subsequent chemical analyses.

Chemical analyses of cations

Sodium and potassium were determined by the flame emission technique which is a rapid and sensitive method for the determination of sodium and potassium. The flame photometer method (Williams and Twine, 1960) using Carl Zeiss flame photometer, was applied

Chemical analyses of anions

Chlorides were measured by $AgNO_3$ titration method as described by Jackson (1958). Sulphates were determined by a turbidemetric method as $BaSO_4$ precipitation by barium chloride and acid sodium chloride requests using a spectrophotometric technique (Black *et al*., 1965).

Table (1): Monthly average records of climatic parameters (temperatures, evaporation rate, relative humidity and wind velocity) according to data of Meteorological Station at Kharga oasis.

Months	Measured parameters					
	Temperature °C			Evaporation rate (ml/day)	Relative humidity (%)	Wind velocity (knots /hr.)
	Maximum	Minimum	Daily Mean			
January	21.2	5.3	12.9	4.67	60	3.3
February	26.5	8.2	17.2	6.05	44	3.1
Marsh	24.7	10.7	17.8	11.6	38	10.4
April	37.2	17.7	27.8	16.09	28	6.2
May	38.1	22.1	30.2	18.29	28	6.1
June	40.4	25.2	33.1	21.03	32	6.2
July	40.3	25.7	33.4	20.75	38	5.2
August	40.6	24.3	33.1	17.72	39	4.8
September	37.9	23.9	30.8	17.29	45	4.2
October	33.3	20.5	26.8	15.92	50	8.3
November	25.8	11.8	18.7	8.73	66	6
December	26.3	11.2	18.5	8.57	61	2.8
Annual average	32.7	17.3	25	13.9	44.1	5.6

Determination of osmotic potential of plant extracts and computation of the actual O.P.

The cryoscopy method of Walter (1949) was used for determination water potential by using Beckman differential thermometer (calibrated at 0.01°C) as illustrated by Slatyer and Mcllory (1961). The total osmotic potential of the cell sap was calculated according to EL-Sharkawi and Abdel Rahman (1974).

Calculation of partial osmotic potential (POP)

The estimation of partial osmotic potential for different ions of plant extracts (chlorides, sulphates, sodium and potassium) was calculated according to the following equation (Kramer and Boyer, 1995):

$$POP\ (of\ ions) = \frac{Ion\ con.(gL^{-1})\ X\ 2.24}{A.Wt\ of\ Ion\ or\ group}\ \text{- MPa}$$

Where A.Wt, is atomic weight; MPa represents water potential.

Statistical evaluation of experimental data

The effects of single factors (season or location) and their interaction (S x L) on the contents of ions in different species were evaluated statistically by the analysis of variance (F test). The relative role of every single factor and their interaction in the total response was determined by using the coefficient of determination (η^2) to indicate the degree of control of the factor on the parameter tested (Ostle, 1963 and Ploxinki, 1969) as applied by EL-Sharkawi and Springuel (1977). A simple linear correlation coefficient (r) between ion concentrations in soils with their equivalents in plants was tested according to Ostle (1963).

RESULTS

Physical and chemical characteristics of the soil

Soil water content (SWC)

Soil water content at the different locations studied was estimated at both soil surfaces (0-5cm) and sub-surface (20-25cm) .The SWC at the surface (Figure, 1) was higher in the winter and reached to1 6.2% of the oven dry soil (site 4), and the lowest value 0.04% was found at site 2 . In the sub-surface soil, the water content showed relatively higher levels in winter at all locations. The highest percentage of SWC (22.7% of the dry soil) was observed at site 8 followed by 17.48% at site 4. At site 1, the water content was exceptionally higher in summer (17.2%) than in winter (13.23%).The lowest percentage (0.4%) was found at site 2 in the two seasons at both soil surface and sub-surface.

From the ANOVA (Table, 2), the effects of season, location and their interaction on SWC were significant at both soil surface and sub-surface soil. The season effect had the dominant role on SWC in the soil surface (η^2=0.44), while the effect of location had the dominant role in soil water content at sub-surface soil (η^2= 0.60).

Total soluble salts (TSS)

In both seasons, the total soluble salts (as % of dry wt.) were higher in surface soil than in sub-surface in most locations (Figure, 1). In soil surface, TSS % during winter was higher than in summer and reached 21.99% at site 8, while the lowest value (0.81%) was found on site 4. In sub-surface soil, the highest value (7.48%) was detected at site 7 in winter; whereas the lowest value (0.08%) was found at site 2 in summer.

Soil pH

The soil pH at all locations was always observed to be alkaline and slightly increased in winter in both soil levels .At the soil surface, the maximum pH value was 8.77 at site 2 and the minimum was 7.24 at site 8. In the sub-surface, the highest value was 8.94 at site 1 and the lowest value was 7.41 at site 8.

Cations

Sodium

Sodium is considered the most abundant cation which has the highest content among investigated cations (Figure, 2). In both seasons, sodium content at soil surface was higher than that at sub-surface soil, except site 8 during summer. At soil surface, the content of Na^+ in winter was higher than that in the summer, where site 8 recorded the highest content of Na^+ (75.83 mg/g. dry wt.) during winter. At sub-surface soil, the content of Na^+ varied from one location to another. At site 7 recorded the highest site 8 observed the highest content (17.35 mg/g. dry wt.) during summer, while site 2 had the lowest Na^+ content (0.03 mg/g. dry wt.) in both seasons.

Potassium

In general, potassium content at surface was higher than that at sub-surface soil, and both soils contained more K^+ during the winter season (Figure, 2). At soil surface, it was observed that site 1 had the highest content of K^+ (9.08 mg/g. dry. wt.) in winter and site 2 gave the lowest content (0.11 mg/g. dry wt.) during summer. The sub-surface soil at site 9 exhibited the highest value of K^+ (6.66 mg/g. dry wt.) in winter whereas the lowest value (0.03mg/g. dry wt.) existed at site 2 in summer.

Anions

Chloride

Apparently, chloride ion was the major anion existing in the soil extract. This was clearly observed at soil sur face (Figure, 3), where in general, the content of Cl^- ion was greater than at sub-surface soil in both season, apparently due to high water evaporation. Moreover, sub-surface accumulates chloride in winter, (probably due to leaching from the surface). The highest content (51.9 mg/g.dry wt.) was found at site 2 and site 9. At sub-surface soil, the highest content of Cl^- (30.34 mg/g.dry wt.) was found at site 8 in summer and at site 7 in winter (Dakhla oasis). The lowest value (0.89 mg/g.dry wt.) existed at sites 2&4.

Sulphate

Sulphate content in soil extract recorded low values at both soil surface and sub-surface soil in the two seasons. Site 1 exhibited the highest content (0.78 mg/g dry wt.) of SO_4^{2-} during summer, and site 8 recorded the highest content (0.6 mg/g.dry wt.) during winter, the lowest content (0.02 mg/g.dry wt.) was observed at site 2 in both seasons at both soil levels (Figure, 3).

Plant water content

Plant water content was determined as a percentage of fresh weight (Table, 4). It was found that water content in most investigated plants was high. In halophytic plants such as *Salsola*, there were no noticeable changes in water content during both seasons at nearly all sites inhabited. *Zygophyllum*, *Cressa* and *Suaeda* showed a slight difference in water content in both seasons. Water content was higher in winter than in summer in most plants. Meanwhile water content was higher in summer than in winter in *Zygophyllum*, and *Salsola*. *Zygophyllum* had the highest average water content (77.16%) followed by *Suaeda* (77.14%).

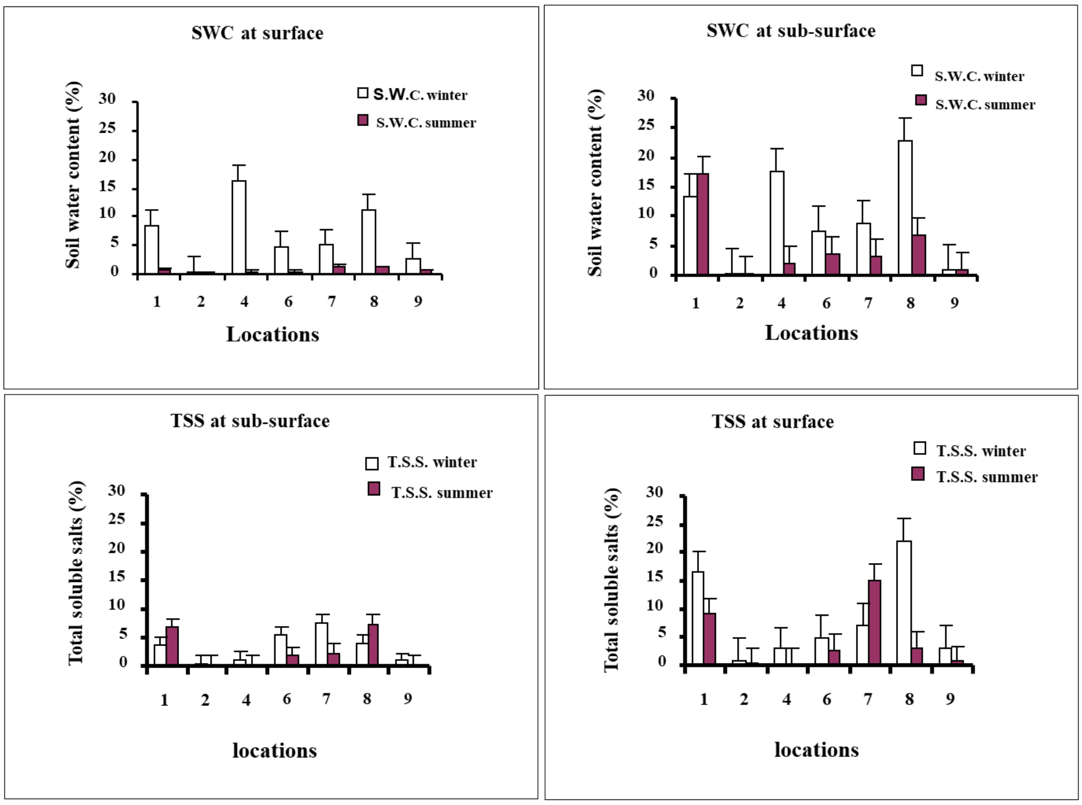

Figure (1): The average percentages of soil water content (SWC), and total soluble salts (TSS) in surface and subsurface soils at different locations during winter and summer.

Table (2): Significance level of the effects of seasons (S), location (L) and their interaction (S x L) on volumetric soil water content at soil surface and at sub-surface soil horizon using ANOVA test.

Soil water content	Source of variance	F value[†]	η^2
At surface (0-5cm)	Seasons	8840.308 **	0.40
	Locations	912.6667 **	0.27
	S x L	969.1567 **	0.29
At sub-surface (20-25 cm)	Seasons	325.8487 **	0.14
	locations	232.1669 **	0.60
	S x L	100.3124 **	0.26

†* Significance level at $p<0.05$ level; ** Significance level at $p<0.01$.

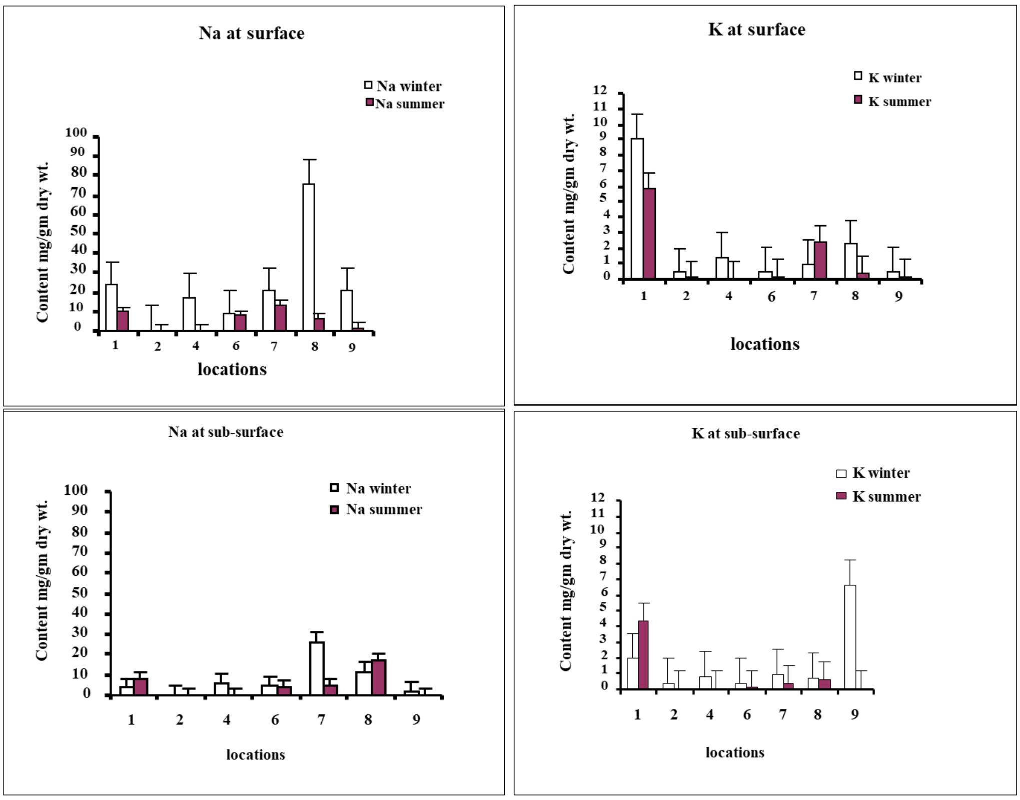

Figure (2): The average content (mg/gm dry soil) of cations Na^+ and K^+ at surface and sub-surface soils at different locations during winter and summer.

Relative water content

Relative water content (RWC) of investigating species at their native location was determined (as a percentage) in winter and summer (Figure, 4). In a xerophyte succulent plant (*Zygophyllum*) there was a high RWC (site 3, 74.3%) during winter and slightly decreased during summer. This means that this species had sufficient water and don't suffer from water deficit. Both *Suaeda*, and Prosopis species had a moderate percentage of RWC (more than 50%), while other plants, such as *Salsola* and *Cressa*, studied species had a low percentage of RWC (less than 50%). This can be explained in such context that such species are suffering from a shortage of water and therefore a water deficit is developed.

Correlation between relative water content and soil water content

In (Table, 3) the correlation between relative water content in investigated plants and soil water content was nonsignificant in *Salsola* species during summer at both soils surface and sub-surface. This may indicate that relative water content is not dependent on the soil water content and the plant has its mean of water conservation (probably through osmotic adjustment). On the other hand, correlation between relative water content and soil water content was significantly positive especially in *Zygophyllum* at both soil levels during both seasons. This may indicate that relative water content is highly dependent on soil water content in such species.

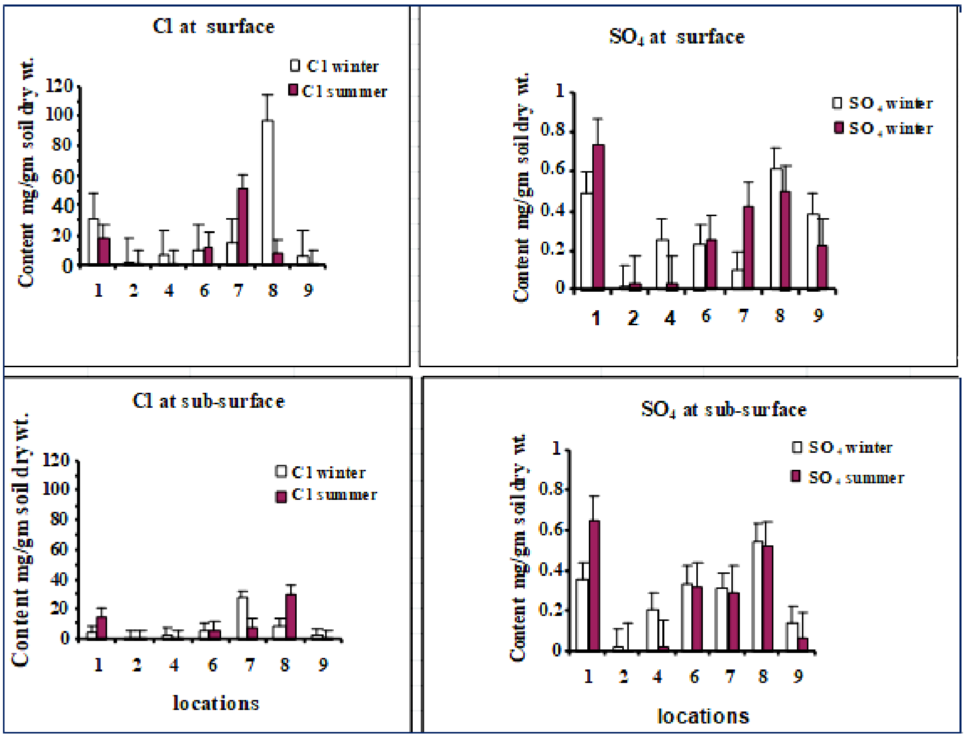

Figure (3): The average content (mg/gm dry soil) of anions (chlorides Cl^- and sulphates SO_4^{2-}) at surface and sub-surface soils at different studied locations during winter and summer.

Table (3): Correlation coefficients (r) values between relative water content (RWC) in investigated plants and soil water content, at the surface and sub-surface in their habitats, during both seasons.

Studied Plant Species	Soil samples			
	At Surface		At Sub-surface	
	Winter	Summer	Winter	Summer
Zygophyllum coccineum	0.934**	0.952**	0.969**	0.931**
Salsola imbricata	-0.362	-0.869**	-0.154	-0.629**
Suaeda monoica	-0.342	-0.644	-0.423	0.493
Cressa cretica	-0.447	0.031	-0.521	-0.296

* Significance level at p<0.05 level; ** Significance level at p<0.01.

Elemental constituents in plant sap

Sodium

Halophytic plants, in general, have higher concentration of sodium (Na^+) compared to succulent species as shown in (Figure, 5). The accumulation of sodium occurred during winter and reached a maximum value of 66.29 mg/ml sap in *Salsola* (as a halophytic species) at site 4. Both *Cressa* and *Suaeda* had higher Na^+ concentration in summer than that in winter. In *Zygophyllum* as a succulent species a relatively low concentration of Na^+ was observed and slightly changed during the two seasons in all studied locations. Exceptionally, the highest Na^+ concentration in the same plant was 20.65 mg/ml sap at site 4 during winter.

Potassium

A general trend in the investigated halophytic plants was their tendency to accumulate K^+ during winter (Figure, 6). Halophytic plants contained the highest amounts of K^+ (33.96 mg/ml sap) e.g. *Salsola*. A succulent xerophyte *Z. coccineum* contained amount of K^+ ranging between 0.83-7.89 mg/ml sap particularly in winter. The accumulation of K^+ varied among all investigated plants during winter. In summer, there were slight changes in K^+ concentration in species at different sites. *Zygophyllum* contained low K^+ concentration during the two seasons. In winter, the highest concentration (7.89 mg/ml sap) was observed at site 2 and the lowest (0.83 mg/ml sap) was found at site 5. In summer, K^+ concentration ranged between 0.86 – 2.08 mg/ml sap.

The ANOVA test in (Table, 4) showed that seasons, locations and their interaction had significant effects on Na^+ and K^+ concentrations in most plants. The effect of seasons on changes in Na^+ concentration had a dominant role in *Suaeda* and *Cressa* (η^2 = 0.69 and 0.79, respectively). Likewise, seasonal effect had a

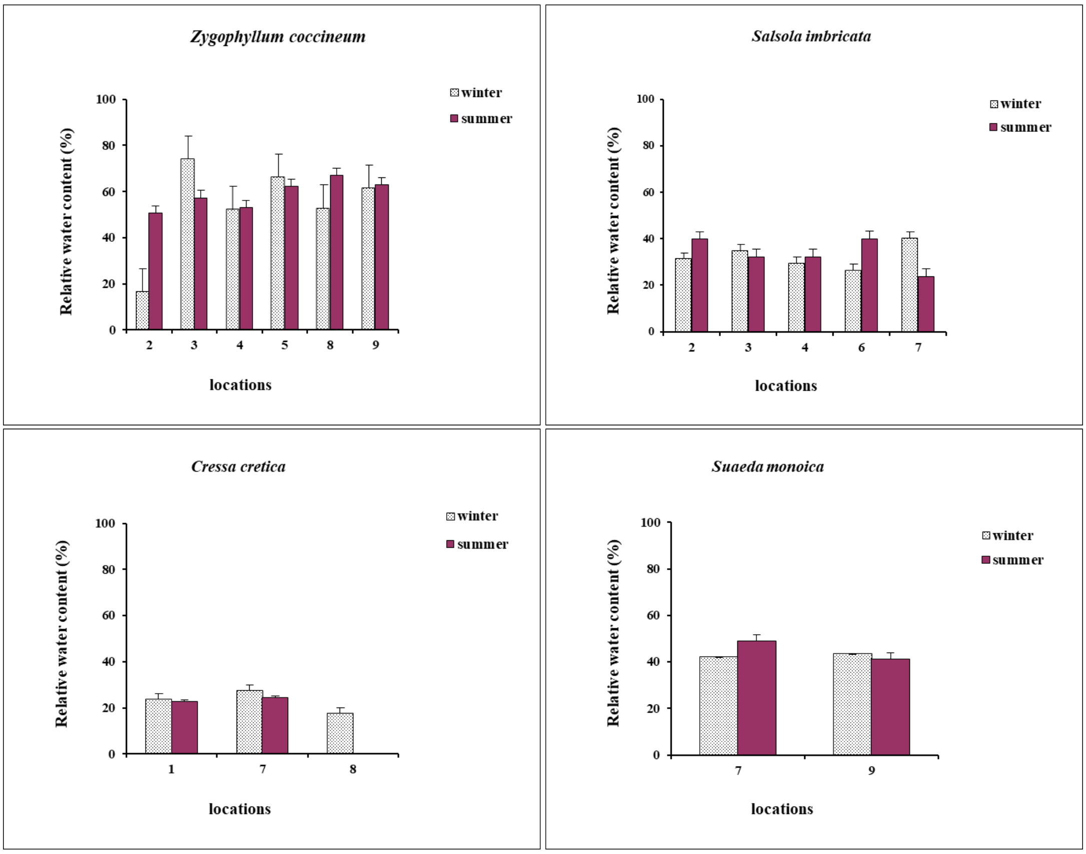

Figure (4): The average percentage of relative water content of investigated species at their locations during winter and summer seasons.

Table (4): Significance level of the effects of seasons (S), location (L) and their interaction (S x L) on cation concentrations (Na^+ and (K^+) of investigated species at Kharga and Dakhla regions, by means of one-way ANOVA test.

Studied Plant Species	**Cation concentration**				
	Source of variance	**F value for Na^+**	η^2	**F value for K^+**	η^2
Zygophyllum coccineum	Seasons	5.51*	0.18	57.12**	0.34
	Locations	1.48	0.24	10.62**	0.31
	S x L	3.50*	0.58	11.82**	0.35
Salsola imbricata	Seasons	10.9**	0.13	36.26**	0.19
	Locations	7.68**	0.38	19.86**	0.42
	S x L	9.85**	0.49	18.33**	0.39
Suaeda monoica	Seasons	7.21*	0.69	40.07**	0.64
	Locations	1.82	0.18	20.66**	0.33
	S x L	1.35	0.13	1.82	0.03
Cressa cretica	Seasons	21.98**	0.79	3.7	0.08
	Locations	2.55	0.09	38.32**	0.87
	S x L	3.34	0.12	2.04	0.05

* Significance level at $p<0.05$ level; ** Significance level at $p<0.01$.

dominant role on k^+ concentration in *Suaeda* (η^2 = 0.64), while the effect of their) interaction (S x L) had an equal share with the effect of seasons on K^+ concentration in the case of *Zygophyllum* (η^2 = 0.35). In parallel, the (S x L) interaction plays a dominant role on Na^+ concentration that detected in *Salsola* and *Zygophyllum* (η^2 = 0.49 & 0.58 respectively). However, the influence of locations on K^+ concentration showed a dominant role (evaluated by η^2 values) in both *Cressa* and *Salsola*.

Anion

Chloride (Cl^-)

Chloride ion concentration in plant sap is shown in (Figure, 7). In general, halophytes had higher Cl^- concentration in succulent species. Also, the concentration of Cl^- in most species was higher in summer than in winter with some exceptions. In summer, the maximum chloride concentration (85.03 mg/ml sap) was found in *Cressa* at site 7 followed by *Suaeda* as halophytic species. While *Zygophyllum* (succulent species) had high Cl^- concentration (31.06 mg/ml sap) was detected at site 8.

Data from F test in (Table, 5) showed a clearly significant role of seasons, locations and their interactions on chloride ion concentration in most species. Change of seasons had a dominant role in affecting chloride ion concentration in most plants and the effect of (S x L) interaction was sub-dominant.

Sulphate (SO_4^{2-})

A general pattern of sulphate accumulation was noticed in the most studied plants during summer (Figure, 8). In winter, halophytes and succulent species had high SO_4^{2-} concentration. *Cressa* at site 1 had the highest SO_4^{2-} concentration (4.45 mg/ml sap). *Zygophyllum* recorded a moderate SO_4^{2-} concentration during both studied seasons that ranged between (0.67– 3.43 mg/ml sap). Also, *Suaeda* species had a moderate range of SO_4^{2-} concentration detected during summer and a low range of SO_4^{2-} concentration in winter. On the other hand, the rest species had a low SO_4^{2-} concentration.

From ANOVA (Table, 5) the effect of either seasons or locations and their interaction on measured SO_4^{2-} concentration was significant in all investigated species. Seasonal variation plays a dominant role in SO_4^{2-} ion in most studied species, except in *Zygophyllum* where (S x L) interaction had a major role ($\eta^2 = 0.47$). Locations had a subdominant role in most plants.

Correlation of investigating plants and ions in surface soil

The data in table (6a) showed that, *Cressa* had a widely dominant positive correlation with K^+ content in both seasons, with Na^+ in summer, with SO_4^{2-} in winter and only one negative correlation with SO_4^{2-} in summer. Moreover, *Salsola* had a dominant positive correlation with Cl^- and Na^+ in summer, with SO_4^{2-}, K^+ in winter. Meanwhile, *Suaeda* had a dominant positive correlation with SO_4^{2-} in summer and with K^+ in both seasons. On the other hand, *Zygophyllum* had a dominant negative correlation with Cl^-, SO_4^{2-} and K^+ in winter and positive correlations with Cl^-, K^+& Na^+ in summer.

Ionic correlation of investigated plants and ions in sub-surface soil

Apparently, *Cressa* had a dominant positive correlation with K^+ in both seasons (Table 6b). A sub-dominant negative correlation was recorded with SO_4^{2-} in summer only. In parallel, *Suaeda* had a dominant positive correlation with K^+ that recorded in summer and a negative correlation in winter. Likewise, *Zygophyllum* had a dominant negative correlation with Cl^-, SO_4^{2-} and K^+ during winter, and a sub-dominant positive correlation with Cl^-, Na^+, and K^+ in summer.

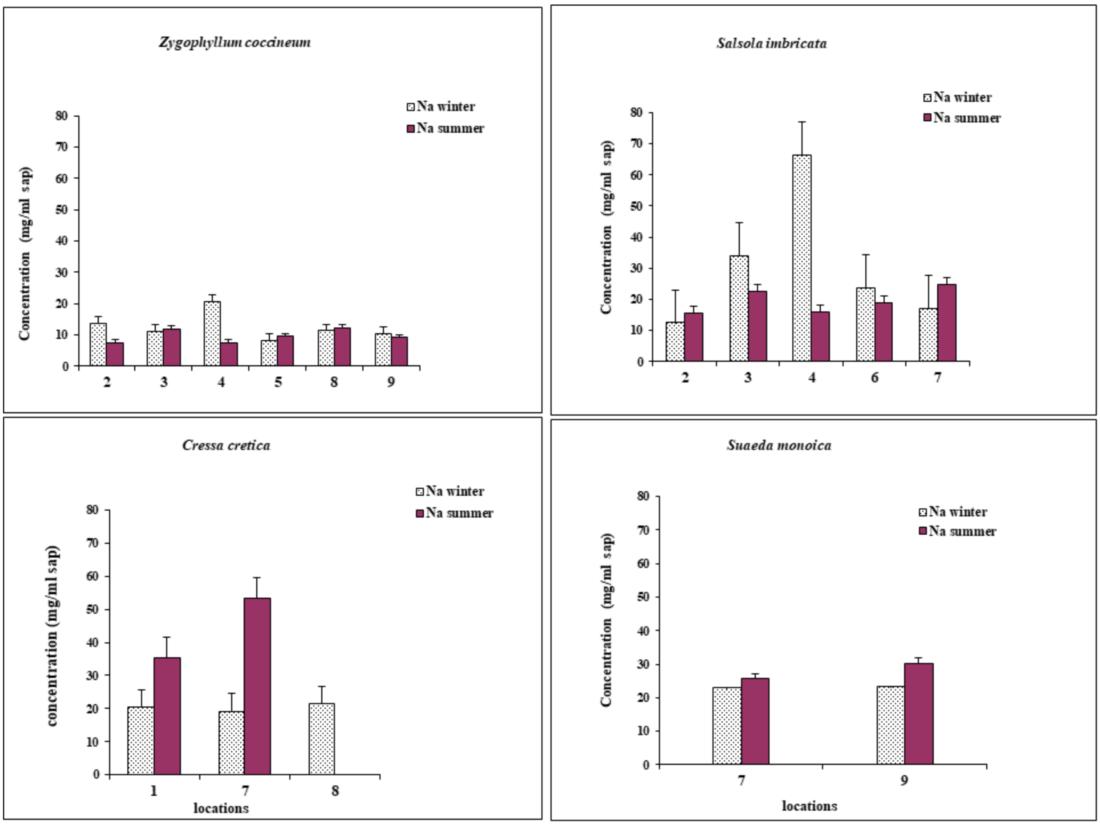

Figure (5):-The average concentration (mg/ml sap) of sodium ion (Na^+) in investigated species at different studied locations during winter and summer seasons.

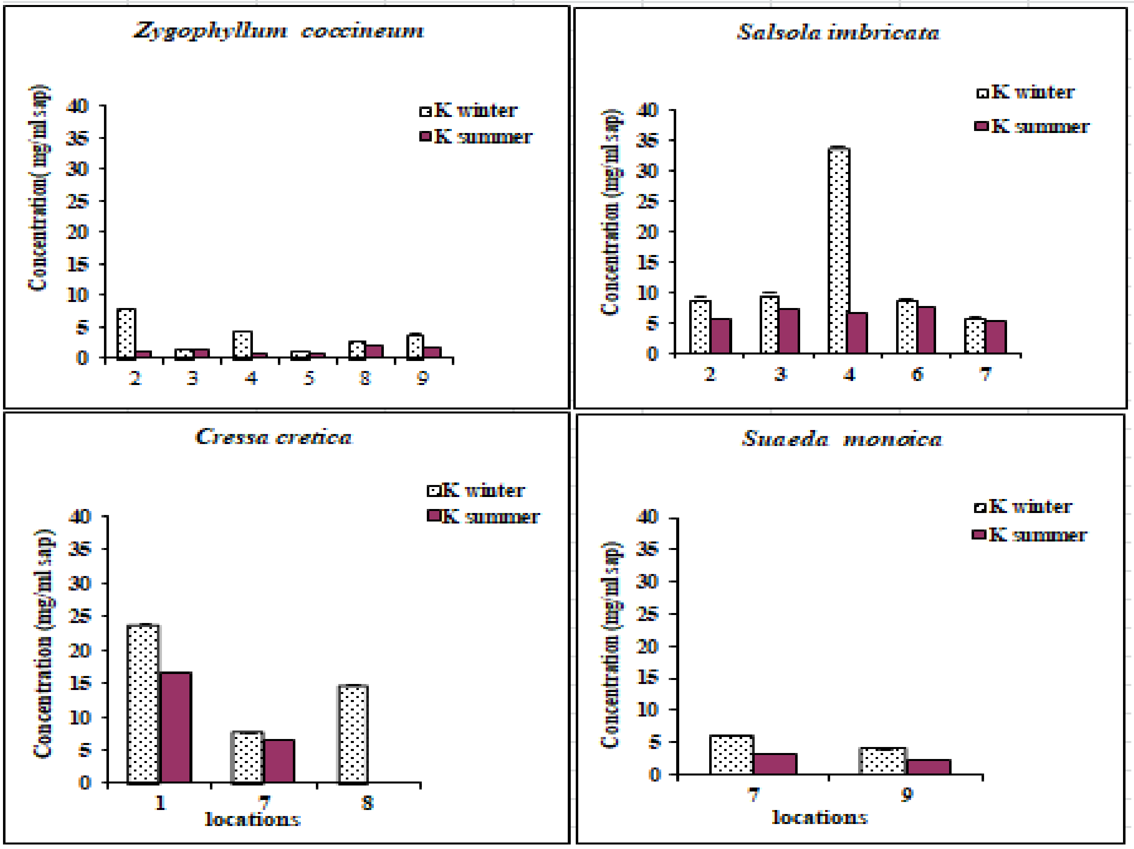

Figure (6): The average concentration (mg/ml sap) of potassium ion (K^+) in investigated species at different studied locations during winter and summer seasons.

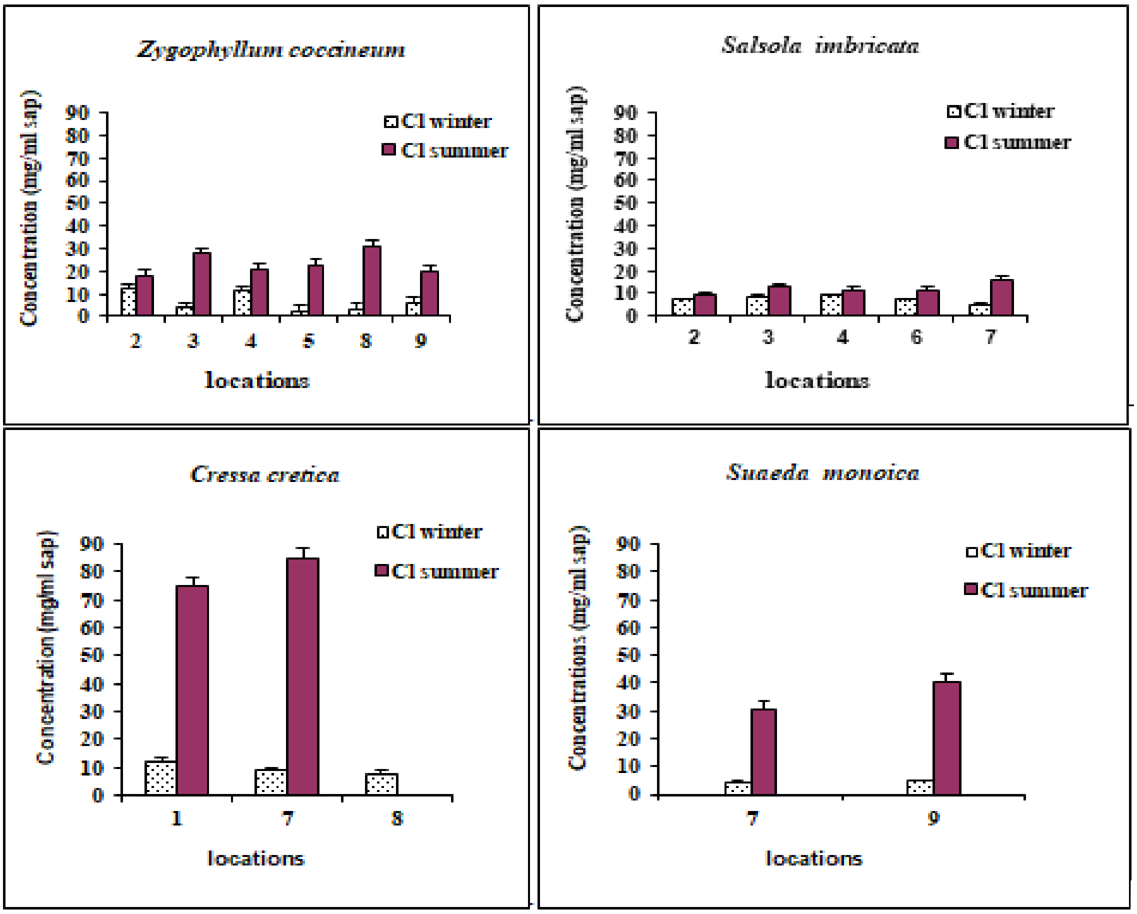

Figure (7): The average concentration (mg/ml sap) of chloride ions (Cl^-) in investigated species at different studied locations during winter and summer seasons.

Table (5): Significance level of the effects of seasons (S), location (L) and their interaction (S x L) on anion concentration (Cl^- & SO_4^{2-}) of investigated species at Kharga and Dakhla regions, using ANOVA test.

Studied Plant species	Source of variance	Anion concentration			
		Cl^-		SO_4^{2-}	
		F	η^2	F	η^2
Z. coccineum	Seasons	264.79**	0.8	11.22**	0.12
	Locations	1.97	0.03	7.49**	0.41
	S x L	11.41**	0.17	8.49**	0.47
S. imbricata	Seasons	124.26**	0.64	73.91**	0.6
	Locations	4.6**	0.1	11.44**	0.37
	S x L	12.5**	0.26	1.06	0.03
S. monoica	Seasons	20.36**	1	80.32**	0.91
	Locations	0.03	0	5.63*	0.07
	S x L	0.04	0	1.92	0.02
C. cretica	Seasons	46.33**	0.99	8.16*	0.88
	Locations	0.11	0	0.31	0.04
	S x L	0.45	0.01	0.77	0.08

*Significance level at $p<0.05$ level; ** Significance level at $p<0.01$.

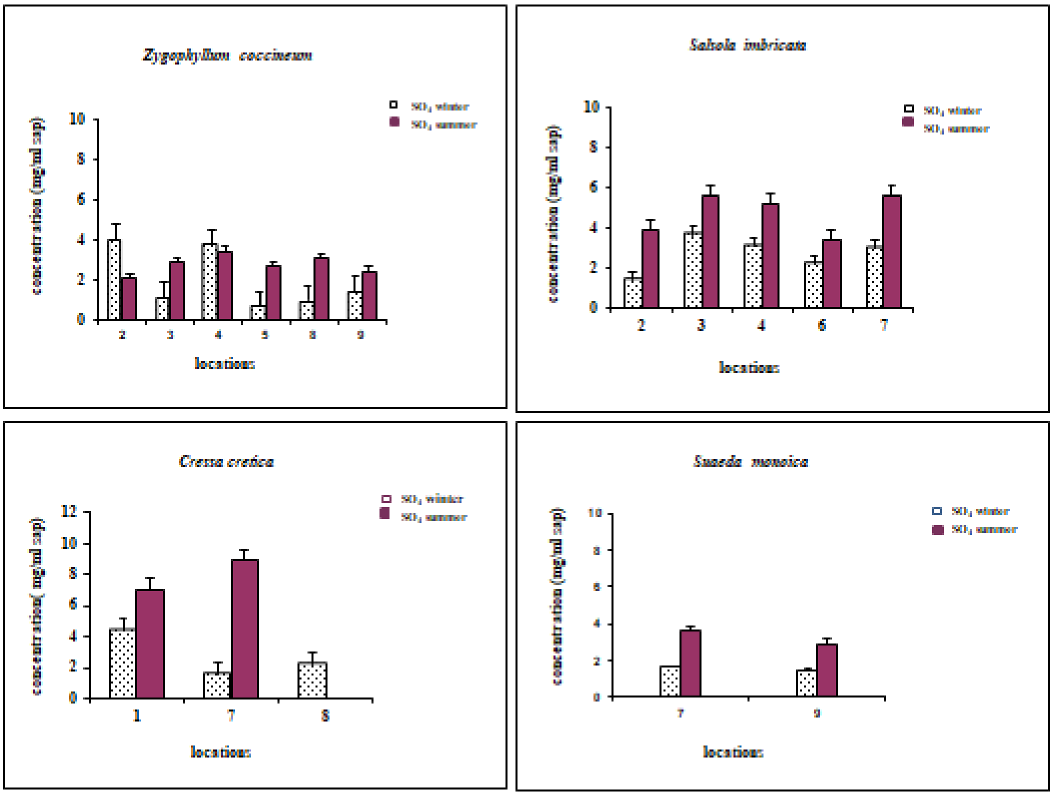

Figure (8): The average concentration (mg/ml sap) of sulphate ions (SO_4^{2-}) in investigated species at different studied locations during winter and summer seasons.

Total and partial osmotic potential

The total osmotic potential (TOP), of investigated plants, revealed the potential ability of the plants for osmotically adjusted during winter (Table 7). *Suaeda* exhibited the highest average TOP during winter. Meanwhile, the average of TOP in *Cressa* was high during summer, whereas the lowest value was observed in *Zygophyllum*.

The major ions that generally affect plant osmotic potential in a high percentage were chloride, sodium, and potassium, whereas the minor ion affecting plant osmotic potential with a low percentage is sulphates. In most investigated species, sodium ion was the major ion in osmoregulation reached 17% - 29% in winter and 19% - 36% during summer. The same was true in the case of chloride ion which had a relatively greater role in osmoregulation. It contributes high % of total osmotic pressure especially in summer season (24% - 42%) but, only (4% - 16%) in winter. This was demonstrated in *Zygophyllum*, *Cressa*, *Suaeda*. Chloride ion had a moderate role in osmoregulation (4% during winter and 13% during summer).

In most investigated species, potassium ion was contributed a relatively moderate percentage of TOP in winter which ranged from 4 to 12%; however, in summer it recorded a lower percentage (2% - 7%). Apparently, TOP of NaCl in *Cressa* was the highest among the investigated species. On the other hand, the osmotic potential ratio of Na^+/K^+ was tended to a maximum in Suaeda in summer. The same was true in case of Cl^- / SO_4^{-2} ratios in both *Suaeda* and *Zygophyllum* for the same season.

Table (6): Correlation coefficient (r) values between ion concentrations of the investigated plants species and their contents at the two sampling sites, surface and sub-surface soil, in their habitats during two seasons.

a- At surface soil

Plant species	Measured ions							
	Cl^-		**SO4 $^{2-}$**		**Na^+**		**K^+**	
	winter	**summer**	**winter**	**summer**	**Winter**	**summer**	**winter**	**summer**
Z. coccineum	-0.941**	0.806**	-0697**	0.246	-0.270	0.873**	--0.74**	0.937**
S. imbricata	-0.457-	0.851**	0.650*	0.226	0.445	0.571*	0.753**	-0.380
S. monoica	-0.475	0.019	-0.361	0.680**	-0.256	- 0.626	0.689*	*0.777*
C. cretica	0.36	0.3	0.859**	-0.943**	0.563	0.698*	0.894**	0.643*

* Significance level at $p < 0.05$; ** Significance level at $p < 0.01$.

b- At sub-surface soil

Plant species	Measured ions							
	Cl^-	**SO_4^{2-}**	**Na^+**	**K^+**	**Cl^-**	**SO_4^{2-}**	**Na^+**	**K^+**
	winter	**summer**	**winter**	**summer**	**winter**	**summer**	**winter**	**summer**
Z. coccineum	-0.927**	0.635*	0.740**	0.279	-0.203	0.841**	0.773**	0.918**
S. imbricata	-0.659**	0.724**	0.520*	-0.025	-0.137	0.438	0.297	-0.286
S. monoica	-0.39	0.158	0.336	0.59	-0.065	-0.641	-0.780*	0.836**
C. cretica	-0.612	-0.235	0.054	0.923**	-0.163	-0.599	0.672*	0.682*

* Significance level at $p < 0.05$;** Significance level at $p < 0.01$.

Table (7): Average values of total osmotic potential (-MPa) and partial osmotic potential ratios of ion participation, represented in percentage (%), in tested species at both seasons.

Plant Species	Season	Measured Parameters							
		TOP†	**Osmotic potential of NaCl**	**POP‡ (%)**	**Cation osmotic**	**AOP†† ratio**	**TOP**	**Osmotic potential of NaCl**	**POP (%)**
		(-MPa)	**(-MPa)**	**Na^+**	**K^+**	**Cl^-**	**SO^{-2}**	**Na^+/K^+**	**Cl^-/SO_4^{-2}**
Z. coccineum	Winter	6.75	1.55	17	6.0	6	1.0	2.83	6.0
	Summer	4.80	2.35	19	2.0	30	1.0	9.5	30.0
S. imbricata	Winter	8.85	2.74	27	6.0	4	1.0	4.5	4.0
	Summer	6.45	2.84	31	6.0	13	2.0	5.17	6.5
S. monoica	Winter	7.65	2.52	29	4.0	4	0.5	7.25	8.0
	Summer	7.20	4.68	36	2.0	29	1.0	18.0	29
C. cretica	Winter	8.25	2.56	23	12.0	8	1.0	1.92	8.0
	Summer	11.4	8.78	35	7.0	42	2.0	5.0	21.0

†TOP, Total osmotic potential; ‡POP, Partial osmotic potential of ion participation; ††AOP ratio, Anion osmotic potential ratio.

DISCUSSION

The water consumption by natural plants depends on the volume of water available to the roots. In this study, soil water content (SWC) was higher in winter than in summer in both surface and sub-surface soils. Apparently, the statistical analyses showed that changes in the season had the dominant effect on soil water content at soil surface, while the location factor had the dominant role on SWC in sub-surface soil. Moreover, the correlation analyses between the relative water content of plants and soil water suggested that the investigated species have a different response to water availability in the soil. *Z. coccineum* (during winter) showed positive correlations at the two soil depth levels. However, *S. imbricata* had negative correlations at both levels during winter. This indicates that, the investigated species may have different mechanisms for water conservation.

Clearly, many plants adjusted osmotically to soil salinity by accumulating ions. Under moderate levels of stress, roots may still actively absorb inorganic ions (potassium, calcium, sodium, magnesium, chloride, and others) from the soil (Amede and Schubert, 2003). This accumulation of mineral ions such as K^+, Na^+, Cl^-, and SO_4^{2-} facilitate osmotic adjustment of plants under atmospheric aridity and soil dryness (Farghali, 1998). Both sodium and chloride ions mostly increase in the vacuolar osmotic concentrations in plants under water stress. In general, the concentration of Cl^- and Na^+ in surface soil studied was greater than in the sub-surface in both seasons, may be due to high water evaporation, while the concentration of SO_4^{2-} is very low at both soil depths in the two seasons. Also, the data indicate that Na^+ is the most abundant cation in soil, having the highest concentration among other cations. The high concentration of Na^+ ions in soils may be probably due to the concentration decrease of other ions (Silberbrush and Ben-Asher, 2001), or may be as a result of interaction with other environmental factors, such as drought, which exaggerates the problems of Na^+ toxicity. Other ions deficiency can occur because increased Na^+ inhibits the uptake of such nutrients by 1. disrupting the uptake of nutrients directly by interfering with transporters in the root plasma membrane, such as K^+-selective ion channels; and 2. inhibiting root growth, by the osmotic effect of Na^+ and because of the detrimental effects of Na^+ on soil structure (Katschnig *et al.,* 2015).

Apparently, the accumulation of different ions in tested species was affected by seasonal variations. In general, the plants had a tendency to accumulate Cl^-, SO_4^{2-}, in summer. In some species especially, *C. cretica*, *S. monoica*, and succulent *Z. coccineum* a high concentration of Cl^- is detected. This means that, the accumulation of Cl^- may occur due to its major osmotic contribution to the solute in the vacuole and its involvement in both turgor and osmoregulation. In the cytoplasm, chloride regulates the activities of enzymes, also it acts as a counter anion and its fluxes are implicated in the stabilization of membrane potential, the regulation of pH gradients and electrical excitability (White and Broadley, 2001). Also, euhalophytes such as *C. cretica*, *S. imbricata* accumulate SO_4^{2-} to maintain their succulence which is usually associated with the increase of sulphate content.

It was found that, high concentrations of K^+, and Na^+ in most studied species were detected during winter. Furthermore, sodium ion was accumulated excess than K^+ accumulation and both cations were found at high levels in halophytes (*Salsola*, *Cressa*, and *Suaeda*). This means that, the increase of Na^+ levels may affect intercellular K^+ accumulation. From our point of view, the accumulation of Na^+ and K^+ found in such halophytes may play an important role in osmotic adjustment. Thus, the ionic osmotic potential in the studied species was mainly related to K^+ followed by Na^+ to tolerate the water stress and higher temperatures. It is found that, such plants could be adapted to drought stress, which might reflect their sensitivity to Na^+ toxicity by an accumulation of K+ ions. Accordingly, the mechanisms of osmotic adju-stments of some xerophytes mainly depending on the accumulation of K^+ and/or sharing with Na^+ ions (Farghali and El- Aidrous,2016). Our findings agree with the work performed by Song *et al*., (2006) that Na^+ may contribute to osmoregulation in *Suaeda* species under both saline and arid environments. However, Glenn *et al*., (1996) pointed out that K^+ is accumulated in response to soil water deficit while Na^+ is accumulated under saline conditions. This indicates that, K^+ ion is not only an essential element for plant growth and development, but also a primary osmoticum in maintaining low water potential for plant tissues (Wang *et al.*, 2004).

In this respect, plants have evolved remarkable mechanisms to regulate K^+ and Na^+ tissue and cellular homeostasis under salt stress (Almeida *et al*., 2017and Zhang *et al.,* 2018). Really, Na^+ is accumulated in vacuoles to maintain low cellular osmotic water potential, whereas most of K^+ is concentrated in the cytosol to maintain the osmotic balance between cytoplasm and vacuole. Therefore, ion accumulation may be one of the most effective strategies for the adaptation of studied species to arid environments.

The ANOVA test clarified that seasons have the dominant role in regulating Cl^- and SO_4^{2--} concentration in most species. This indicates that sulphate taken up by the plant, which is in surplus to immediate requirements for growth, is stored in the vacuole. This means that, the effectiveness of mobilization of this vacuolar sulphate pool varies, and may reflect species differences or the ability of remobilization processes to keep pace with growth rates (Hawkesford, 2000). The seasonality has the dominant effect on Na^+, and K^+ concentration in halophytes *S. monoica* and *C. cretica,* whereas both ions were affected by the interaction (S x L) in the case of *Z. coccineum*. Also, halophytes investigated had lower osmotic potential than xerophytes. The total osmotic potential was lower in summer than in winter in the majority of plants.

In general, the osmotically adjusted species mainly accumulate solutes depending on the seasonal and species variations. Hence, the ionic osmotic potential (IOP) and/or the relative water content (RWC) in plants are related to solutes such as Cl^-, Na^+, and K^+ in to tolerate the water stress under hot air conditions. However, the significant negative correlation between both Ionic osmotic potential and RWC probably means that the concentration of the solutes decreases during the increase of RWC and vice versa. Whereby, such plants can be adapted to drought injury and thus differ in their mechanisms of osmotic adjustment in response to prevailing stresses, e.g. in *Z. coccineum* and *S. imbricata*, which may reflect their sensitivity to the toxic ions (Na^+ & Cl^-) and alternatively by increased binding of the water molecule (soluble proteins) to overcome the water loss. Finally, it is vital to differentiate solutes accumulation as a concentration effect from active osmotica using cell water volume of control plants before considering solute concentration as selection criteria for breading drought resistance varieties crops (Amede and Schubert, 2003).

CONCLUSION

The data hitherto, may be concluded the followings: 1.The greater absorption ratio attributed to increased Na^+ in the halophytic species indicate its capability to tolerate Na^+ toxicity; 2. The selectivity of Na^+, K^+, and Cl^- positively decrease the ionic water potential in plants, particularly in the hot season. The ionic osmotic water potential of Na^+/K^+ and Cl^- / SO_4^{-2} ratios are the promising screening tools for salt tolerance in studied species and halophytes in general.

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تقييم الاسموزية الايونية فى النباتات الصحراية الملحية العصيرية وغير العصيرية القاطنة للواحات الحارة

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الملخص العربى

هذه الدراسة تم تنفيذها فى واحتى الخارجة والداخلة فى الصحراء المصرية الغربية. الانواع النباتية قيد البحث اساسا لها أصول بيئية وصور حياة مختلفة لايجاد دلائل مقارنة لوسائل الانضباط الايونى بينها. وقد تم تحليل أيونات العناصر الذائبة فى الماء لكل من التربة والنباتات. كذلك تم حساب الجهد المائى الاسموزى والشقوق الايونية فى النباتات. وقد أظهرت الدراسة ان النتائج التي تم الحصول عليها تقسر أستمرار النباتات الملحية فى انضباطها الاسموزى نتيجة تراكم الايونات، وهذا معتمد على الاختلافات الموسمية والنوع النباتى والذى ينتمى الى أيونات الكلوريدات، الصويوم والبوتاسيوم. كما كان للموسمية أو الموقع الدور السائد فى التأثير على تركيزات أيونات الصوديوم، البوتاسيوم، الكلوريدات والكبريتات فى نباتى المليح والسويدة (نباتات ملحية)، بينما فى نبات الرطريط (صحراوى) كانت الايونات متأثرة بالتفاعل التبادلى بين الموسمية ومكان الدراسة (الموقع). وقد تم مناقشة الجهد المائى الاسموزى لنسب أيونات الصوديوم/ البوتاسيوم و الكلوريدات/الكبريتات لتحمل النباتات، قيد الدراسة، للملوحة.