

Seasonal changes of inorganic and organic osmolyte content in three endemic *Limonium* species of Lake Tuz (Turkey)

Gökçen BAYSAL FURTANA^{1*}, Hayri DUMAN¹, Rukiye TIPIRDAMAZ²

¹Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

²Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey

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Abstract: The levels of inorganic (Na^+ , K^+ , Ca^{+2} , Mg^{+2} , Cl^-) and organic (proline, carbohydrates) osmolytes and quaternary ammonium compounds were measured in 3 endemic *Limonium* Mill. species [*L. lilacinum* (Boiss. & Bal.) Wagenitz, *L. iconicum* (Boiss. & Heldr.) Kuntze, and *L. anatolicum* Hedge] of Lake Tuz in central Anatolia, Turkey. Samples were collected between May and October from plants growing in their natural habitats. Soil samples also were taken from the plant root zone at the time of plant sampling. The results of pH, electrical conductivity (EC) values, and mineral composition in the soil showed seasonal changes. The highest EC and soil Na^+ concentrations were found in the autumn. The seasonal changes in the osmolyte concentrations of plants were found to be dependent on soil properties such as EC, anions, and cations. The investigated plants exhibit different abilities to accumulate proline, inorganic ions, soluble carbohydrates, and quaternary ammonium compounds. Proline, ion, and carbohydrate concentrations were found to be negatively related to EC, Na^+ , and Cl^- values of soil. These results show us that each species has its own adaptation mechanism to survive in saline soil. The findings of this investigation are the first reported results based on for these 3 endemic species of *Limonium* located at Lake Tuz, Turkey.

Key words: *Limonium*, halophyte, Lake Tuz, osmolytes, seasonal changes

1. Introduction

Salinity of soils is one of the major limiting factors that adversely affect growth and development of plants (Hamdy et al., 2002) and agricultural productivity (Vera-Estrella et al., 2005). Strategies of plant adaptation to extreme factors depend on the plant genotype, determining morphological, biochemical, and physiological mechanisms for plant growth under previously unfavourable conditions (Radyukina et al., 2007). Plants are classified as glycophytes (salt-intolerant) or halophytes (salt-tolerant) according to their capacity to grow on a high-salt medium. Among all terrestrial plants, halophytes comprise only 2% of species. The remaining 98% of all species are glycophytes displaying a low tolerance to salinity. However, glycophytes are a heterogeneous group comprising sensitive and relatively tolerant species (Radyukina et al., 2007). Halophytes are distributed in coastal and inland saline habitats such as deserts, salt marshes, and coastal areas throughout the world (Ungar, 1991; Raven et al., 1992). The adaptation of halophytes has been known to vary among species (Waisel, 1972; Fahn, 2000; Kim et al., 2002; Adebooye et al., 2012; Fakhireh et al., 2012); plants adapted successfully to and maintained in high soil salinity generally develop certain

structures to either tolerate or survive such environments. Halophytes do not only tolerate salinity but can also be stimulated by salinity (Flowers et al., 1977; Koyro, 2006). Halophytes in saline conditions usually accumulate inorganic ions in vacuoles to decrease cell water potential, because the energy consumption from absorbing inorganic ions is far less than from synthesising organic compounds (Munns et al., 2002). Salt tolerance in plants is a complex phenomenon involving morphological, physiological, and biochemical processes.

Guvensen et al. (2006) revealed that more than 300 halophytic taxa are situated in Turkey. These belong to 150 genera and 40 families. These include the species with high salt tolerance belonging mainly to the families Chenopodiaceae, Plumbaginaceae, Zygophyllaceae, and Frankeniaceae.

Halophytic plants are used for food, fibre, animal feed, medicine, etheric oil, and gum production (Somers, 1982). Although these uses have not been evaluated economically in Turkey, halophytes can at least be used for biological desalination and reclamation of saline-alkaline habitats. *Limonium* Mill. species [*L. iconicum* (Boiss. & Heldr.) Kuntze and *L. lilacinum* (Boiss. & Bal.) Wagenitz] are

* Correspondence: gbaysal@gazi.edu.tr

labelled locally by the Turkish people as “eşek kulağı” and “deve kulağı”. While the fresh leaves of 2 species of *Limonium* (*L. iconicum* and *L. lilacinum*) are eaten by animals, the dried flowers are used locally as a drug by the Turkish people. The aesthetic and natural recreational values of halophytes are very well known in Turkey.

Limonium lilacinum (Boiss. & Bal.) Wagenitz, *L. iconicum* (Boiss. & Heldr.) Kuntze, and *L. anatolicum* Hedge are endemic perennial herbs in the Irano-Turanian phytogeographic region and are spread on the inland salt steppes of central and western Anatolia, Turkey. The ability to grow these species in their native habitat would preserve their populations and reduce grazing pressure. Although there are some studies about the halophyte vegetation of the region, there are not enough studies about the salinity tolerance mechanisms of these halophytes. The main goals of the present study are: to research the ecophysiology of 3 endemic *Limonium* species, to show the investigated parameters’ (organic and inorganic osmolytes) role in the adaptation mechanism of these plants against salt, and to show seasonal variations in the investigated parameters (organic and inorganic osmolytes) of 3 halophytic plants.

2. Materials and methods

Central Anatolia is rich in terms of lakes. One of them, Lake Tuz (Turkish: Tuz Gölü; vicinity of Konya-Ankara-Aksaray provinces), is surrounded by salty marshes. In the arid areas around this lake, there are halophytic vegetation zones with changing physiognomy and floristic compositions. In 2001, Lake Tuz was declared a Specially Protected Area, including all of the lake’s surface and surrounding water beds and some of the important neighbouring steppic areas. The salty marshes in the region constitute the richest areas of these provinces in terms of endemism (especially Lake Tuz).

Lake Tuz is a saline lake occupying a huge area in the arid central plateau of Turkey, about 105 km north-east of Konya, also neighbouring Niğde and Ankara provinces. It is the second largest lake of Turkey, lying at an elevation of 905 m as a tectonic lake. Despite its huge area (1500 km²), for most of the year it is very shallow (between 0.5 to 1 m

deep), especially during the dry summer months when the water evaporates in huge quantities, leaving a thick crust of salt on the surface up to 30 cm. It is not only the biggest salt lake of Turkey, but one of the biggest in the world, as well. Although salinity level changes with seasonal fluctuations, this lake’s water is extremely saline, with a salt rate of 32.4%, and its density is 1.225 g/cm³. Rainfall in the surrounding area is as low as 250 mm per year. The highest temperatures are seen in June and September when the rain is minimal. The arid season, which begins at the end of June, continues for 3–4 months. The area is under the influence of a semiarid cold Mediterranean climate. Seventy-eight per cent of the soils in the basin are characterised as salty and alkaline. Consequently, they behave as suitable habitats for halophytic higher plants and for a few glycophytic ecotypes.

The family Plumbaginaceae is represented by 52 species and subspecies belonging to 6 genera in Turkey. *Limonium* is a member of the family Plumbaginaceae. *Limonium* is represented with 22 taxa (5 sections) in Turkey. Seven of these are endemic to Turkey (Davis et al., 1982; Davis et al., 1988; Evliyaoğlu et al., 2008; Özhatay et al., 2009; Özhatay et al., 2011). Section, flowering time, habitat, altitude, and location of *L. iconicum*, *L. lilacinum*, and *L. anatolicum* are given in Table 1.

Three main locations around Lake Tuz were studied, as shown in Figure 1. These locations were designated as 1 (Şereflikoçhisar), 2 (Aksaray-Eskil), and 3 (Cihanbeyli-Gölyazı). Samples of the plants and soil were collected from these locations. Plant and soil samples were collected from the 3 locations on 21 September 2007, 6 June 2008, 4 July 2008, 25 July 2008, and 10 October 2008. In order to assess the results seasonally, 6 June 2008, 4 July 2008, and 25 July 2008 are specified as summer, and 21 September 2007 and 10 October 2008 are specified as autumn.

Soil samples were collected as a profile at a depth of 0 to 15 cm from each locality. The physical and chemical properties of soil including pH of the soil extract, electrical conductivity (EC), and mineral content (Na⁺, K⁺, Ca²⁺, Mg²⁺, HCO₃⁻, Cl⁻, SO₄²⁻) were estimated using a saturation paste (Tuzuner, 1990).

Table 1. Section, flowering time, habitat, altitude, and location of *Limonium iconicum*, *L. lilacinum*, and *L. anatolicum*.

Section	Species	Flowering time	Habitat and altitude	Location
<i>Limonium</i>	<i>Limonium iconicum</i>	June–September	Inland salt steppe, 900–1040 m	1, 2, 3
<i>Sphaerostachys</i>	<i>Limonium lilacinum</i>	June–September	Saline places inland, 900–1200 m	1
<i>Sarcophyllum</i>	<i>Limonium anatolicum</i>	June–September	Saline places inland, 900–1000 m	1, 2



Figure 1. A map of Lake Tuz showing the study locations: L1 (Location 1: Şereflikoçhisar), L2 (Location 2: Aksaray-Eskil), and L3 (Location 3: Cihanbeyli-Gölyazı).

Plants were collected when they reached their full development. Diagnosis of plant species was given according to the regional flora of Turkey (Davis et al., 1982).

The aerial parts of plants (shoots, leaves, flowers) were washed before being dried out in an oven (60 °C for 4 days) and then crushed into a fine powder with a mortar and pestle. Inorganic (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and Cl^-) ions were determined in extracts prepared according to the method of Prakash and Prathapasenan (1988). In 5 mL of 0.1 N HNO_3 , 100 mg of the dried powder was suspended and the mixture was placed in a boiling water bath for 60 min. The suspension was then cooled and the supernatant was decanted and filtered. The process was replicated 2 more times, and the 3 filtrates were pooled. These crude extracts were used in the assay described below. Na^+ and K^+ were determined by flame photometry (Eppendorf), and Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrophotometer (Varian SpectrAA 5). Cl^- was detected with a Buchler-Cotlove chloridometer.

Proline content was measured according to the method of Bates et al. (1973). Proline was extracted from leaf samples of 100 mg dry weight with 2 mL of 40% methanol. One millilitre of extract was mixed with 1 mL of a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3:2, v/v) and 25 mg of ninhydrin. After 1 h incubation at 100

°C, the tubes were cooled and 5 mL toluene was added. The absorbance of the upper phase was spectrophotometrically determined at 528 nm.

Soluble carbohydrates were extracted in ethanol (80%). Glucose and fructose were analysed by reacting 0.5 mL of extract with 2.5 mL of freshly prepared anthrone (150 mg anthrone + 100 mL H_2SO_4) and placed, for glucose, in a water bath at 95 °C for 5 min or, for fructose, in a water bath at 40 °C for 30 min (Halhoul & Kleinberg, 1972). After cooling, the absorbance at 625 nm was determined in a Shimadzu UV-1700 spectrophotometer.

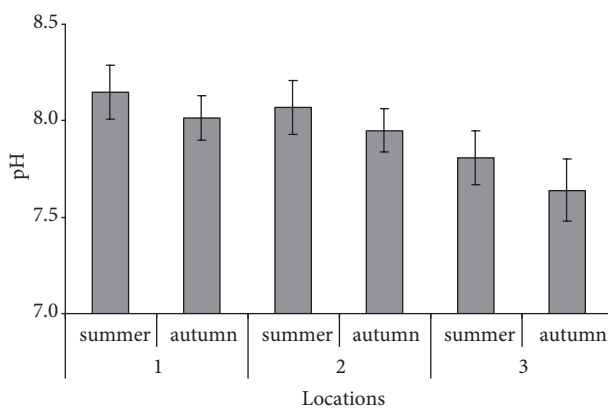


Figure 2. Seasonal pH values of soil samples collected from locations of the investigated species.

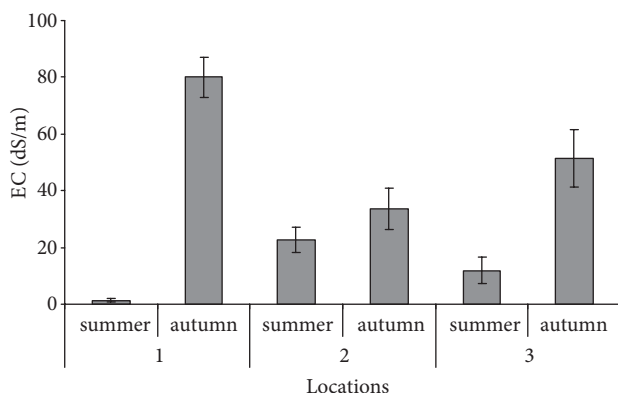


Figure 3. Seasonal EC values of soil samples collected from locations of the investigated species.

Methylated quaternary ammonium compounds were quantified through ¹H-NMR analysis according to the method of Magne and Larher (1992). Dry powders (around 40 mg) were incubated in 1.5 mL of 96% ethanol at 95 °C until the complete evaporation of the ethanol and the dry residues were resuspended with 1.5 mL of deionised water. Aliquots of crude extracts were then freeze-dried (Alpha 2-4 LO). Just before analysis, residues were redissolved in 0.7 mL of D₂O (99.9% deuterium) containing *tert*-butanol (0.5 mM) as an internal standard. The butanol was used as a reference both for the chemical shift (1.2000 ppm) and the quantification of the signals. ¹H-NMR spectra were recorded on a NMR spectrometer (Bruker 400 MHz AV). The processing of the spectra was carried out using XWIN-NMR.

All analyses were carried out with 3 replications. The StatGraphics Centurion XV statistical packet program was used for analysis of the data. The results were subjected to analysis of variance (ANOVA) and compared with multiple range tests at the 95% least significant different. Relations between plants or between plants and soils were analysed with correlation tests.

3. Results

Soil samples were taken from the plant root zone at the time of plant sampling. The physical and chemical properties of soil, including pH of soil extract, EC, and mineral content (Na⁺, K⁺, Ca²⁺, Mg²⁺, HCO₃⁻, Cl⁻, and SO₄²⁻), were measured. The results obtained from the study are shown in Figure 2, Figure 3, and Table 2.

The results of the soil show significant seasonal changes in the pH, EC values, and mineral compositions. Results of soil reaction values are slightly alkaline in the 3 different localities. The values of pH vary between 7.62 and 8.17 in the 3 studied locations (Figure 2). Seasonal changes in pH are not significant. However, the differences between location 1 and location 3 are considerable. The mean values of the EC and soil Na⁺ concentrations change among the 3 localities and range from 1.34 to 138 dS/m (Figure 3) and 5.12 to 640.94 mEq/L (Table 2), respectively. The differences between location 1 and location 2 are important both in summer and autumn. The highest EC and soil Na⁺ concentrations are found in the autumn. Significant seasonal changes are observed in EC and soil Na⁺ concentrations. In general, according to EC and soil Na⁺ values, locations can be classified as extremely saline. The cations of the studied locations are dominated by Na⁺, followed by Mg²⁺ and Ca²⁺, while the K⁺ ion is the least dominant. All the analysed cations behave with the same trend, with the highest values attained in the autumn (Table 2). The values of soil concentrations of Cl⁻ and SO₄²⁻, which vary among the different 3 localities, are not significant. The highest soil Cl⁻ and SO₄²⁻ concentrations are found in the autumn (Table 2).

Surface soil salinity increases in the autumn. Seasonal variations in Na⁺, Mg²⁺, Cl⁻, and SO₄²⁻ concentrations tend to follow the same seasonal pattern as that of EC. The high salt content measured in the autumn is the result of upward salt movement in the soil due to the dry weather conditions.

The plants under investigation from different times exhibit different abilities to accumulate proline, inorganic

Table 2. Seasonal pattern of soil samples (Na⁺, K⁺, Mg²⁺, Ca²⁺, Cl⁻, HCO₃⁻, SO₄²⁻) collected from locations of the investigated species.

Locations	Season	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Mg ²⁺ (mEq/L)	Ca ²⁺ (mEq/L)	Cl ⁻ (mEq/L)	HCO ₃ ⁻ (mEq/L)	SO ₄ ²⁻ (mEq/L)
1	Summer	5.12 ± 3.15	0.76 ± 0.09	3.39 ± 2.37	5.82 ± 4.30	6.24 ± 4.21	2.54 ± 0.09	6.10 ± 19.17
	Autumn	640.94 ± 134.02	30.07 ± 5.26	215.85 ± 36.30	31.91 ± 14.15	426.52 ± 176.26	8.99 ± 5.14	482.22 ± 319.68
2	Summer	146.33 ± 31.15	14.33 ± 3.90	99.09 ± 24.37	12.19 ± 4.30	185.40 ± 46.21	3.15 ± 0.09	72.38 ± 19.17
	Autumn	290.29 ± 134.02	17.78 ± 5.26	110.19 ± 36.30	26.51 ± 14.15	208.11 ± 176.26	7.08 ± 5.14	228.96 ± 192.68
3	Summer	81.67 ± 31.15	5.84 ± 3.90	31.04 ± 24.37	13.72 ± 4.30	53.55 ± 46.21	2.88 ± 0.09	69.56 ± 19.17
	Autumn	459.45 ± 189.53	4.77 ± 3.43	159.02 ± 51.34	12.11 ± 10.01	46.23 ± 24.27	31.13 ± 7.27	559.91 ± 452.10

ions, soluble carbohydrates, and quaternary ammonium compounds. The contents of the plants show variations (Table 3; Figure 4). In the autumn, a decrease is observed in the concentration of Na^+ in *L. lilacinum*, while an increase is shown in *L. iconicum*. Moreover, in *L. anatolicum* the concentration of Na^+ does not change significantly during the period of investigation, nor does the Cl^- concentration. In *L. anatolicum*, the concentration of K^+ increases in the autumn, but it does not change significantly in *L. iconicum* and *L. lilacinum*. In the autumn, concentrations of Ca^{2+} and Mg^{2+} decrease in *L. anatolicum* and *L. iconicum*, but their levels do not change significantly in *L. lilacinum* (Table 3). Significant differences of *L. anatolicum* from *L. lilacinum* and *L. iconicum* are seen in summer. A negative correlation between the plant Na^+ concentration and Mg^{2+} and Ca^{2+} is observed.

The investigated species show different abilities to accumulate proline (Table 3). In the autumn, a decrease is observed in the proline content in *L. anatolicum* and *L. lilacinum*, while an increase is found in *L. iconicum*. The content of proline shows significant negative correlations with soil EC, Na^+ , K^+ , Mg^{2+} , and Cl^- concentrations. The total amount of soluble carbohydrates (glucose and fructose) is highest in the summer in all 3 species (Table 3). Glucose levels decrease strongly in the autumn. The investigated plants show different abilities to accumulate fructose. A decrease is observed in *L. iconicum*, while an increase is seen in *L. lilacinum*. The level of fructose is found to be stable during the period of investigation in *L. anatolicum*. Significant seasonal changes are observed in amounts of glucose and fructose. In general, proline, ion, and carbohydrate concentrations are proven to be negatively related to EC, Na^+ , and Cl^- values of soil.

The 3 *Limonium* species accumulate different amounts of quaternary ammonium compounds, especially β -alanine betaine and choline-O-sulphate (Figure 4). The levels of β -alanine betaine and choline-O-sulphate change in parallel: the highest level of β -alanine betaine and choline-O-sulphate are measured in the summer,

and then they decrease in the autumn. In *L. iconicum*, the levels of β -alanine betaine and choline-O-sulphate are highest in the summer (Figure 4). Depending on the season and the plant, the level of β -alanine betaine shows significant differences. The seasonal changes in the osmolyte concentrations are proven to be dependent on soil properties (such as EC, anions, and cations). Not only does the nature of osmolyte show species-specific differences, but so does the seasonal pattern of osmolyte accumulation.

4. Discussion

Soil characteristics are the main factors influencing plant growth and the distribution of plant communities. Several researchers have proven that there is a relationship between vegetation and soil features (Liangpeng et al., 2007; Morsy, 2007; Youssef & Al-Fredan, 2008).

Several studies (Ungar, 1991; Gul et al., 2001) have shown that variations in the soil salinity content during the year may be reflected by changes in root or shoot ion content at different times during the phenological development of plants. An important aspect of the variations in soil salinity is the relation between salt concentration and electrical conductivity (Álvarez et al., 2000). The locations under investigation as saline habitats show salinities ranging from 1.34 to 138 dS/m. Seasonal variations in the soil's salt concentrations tend to follow the same seasonal pattern as that of EC (Figure 3; Table 2). The highest EC and soil salt concentrations are found in the autumn, which is the result of upward salt movement in the soil due to the dry weather conditions. In general, according to EC and soil Na^+ values, locations can be classified as extremely saline. Soils in these locations accumulate more Na^+ , SO_4^{2-} , and Cl^- than other cations like K^+ , Ca^{2+} , Mg^{2+} , and HCO_3^- (Table 2).

Salinity is a widespread problem leading to decreasing yields in irrigated agriculture. The standard approach to this issue would be to increase the salt tolerance of crop species, but the current success of that option

Table 3. Seasonal variations in fructose, glucose, proline, and inorganic ion (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^-) contents of *Limonium iconicum*, *L. lilacinum*, and *L. anatolicum*.

Species	Season	Glucose (mg/g DW)	Fructose (mg/g DW)	Proline ($\mu\text{mol/g KA}$)	Na^+ (mg/g DW)	K^+ (mg/g DW)	Mg^{2+} (mg/g DW)	Ca^{2+} (mg/g DW)	Cl^- (mg/g DW)
<i>Limonium iconicum</i>	Summer	134.29 \pm 41.31	156.35 \pm 35.45	15.41 \pm 1.40	28.50 \pm 4.76	14.00 \pm 2.54	22.57 \pm 6.68	9.03 \pm 0.63	1.35 \pm 0.11
	Autumn	3.80 \pm 2.46	2.12 \pm 1.23	19.40 \pm 6.10	32.55 \pm 9.80	15.23 \pm 2.98	11.45 \pm 8.07	3.00 \pm 1.07	1.62 \pm 0.52
<i>Limonium lilacinum</i>	Summer	132.41 \pm 50.59	96.98 \pm 43.41	16.80 \pm 1.72	36.75 \pm 5.83	12.75 \pm 3.88	23.65 \pm 8.19	6.40 \pm 0.77	1.59 \pm 0.13
	Autumn	80.07 \pm 40.25	124.39 \pm 46.99	2.25 \pm 8.63	18.00 \pm 13.87	10.50 \pm 4.22	52.50 \pm 11.42	6.80 \pm 4.83	1.34 \pm 0.74
<i>Limonium anatolicum</i>	Summer	86.60 \pm 71.54	44.73 \pm 31.40	27.01 \pm 2.43	31.50 \pm 8.24	9.90 \pm 1.95	68.90 \pm 11.58	37.50 \pm 1.09	1.92 \pm 0.19
	Autumn	34.80 \pm 23.24	38.76 \pm 27.13	9.87 \pm 4.98	31.00 \pm 8.01	14.80 \pm 2.44	19.87 \pm 6.59	11.23 \pm 7.41	1.88 \pm 0.43

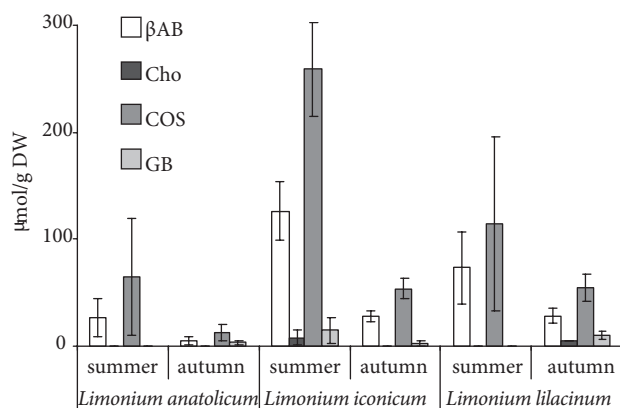


Figure 4. Seasonal variation in glycine betaine (GB), choline-O-sulphate (COS), choline (Cho), and β -alanine betaine (β AB) contents of *Limonium iconicum*, *L. lilacinum*, and *L. anaticum*.

remains limited. An alternative approach is to make use of those plants that have high tolerance for salinity, i.e. halophytes. These plants are able to grow normally in saline environments (Yasseen & Abu-Al-Basal, 2008). In halophytes, a complex of characteristics required for salt tolerance is already present, and all that is needed is to make optimal use of these species (Hamed et al., 2004). Salt tolerance of halophytes is a multigenic trait; the same is true for nonhalophytes (Flowers, 2004).

The aim of this study was to investigate role of the organic and inorganic osmolytes in the adaptation mechanism of these plants against excess salt. We have also researched seasonal variations in the investigated parameters of these halophytic plants.

Mechanisms of salt tolerance can vary by halophyte species. It can be achieved by a combination of osmotic adjustment of the cytoplasm, salt extrusion across the plasma membrane, or salt accumulation in vacuoles (Zhu, 2001; Mimura et al., 2003). Although most halophyte species control the accumulation of inorganic ions as a basic mechanism to adjust their internal tissues' osmotic potential against external salinity, they differ widely in the extent to which they accumulate inorganic ions (Glenn et al., 1996). The investigated species accumulate different amounts of inorganic ions such as Na^+ and K^+ (Table 3). The ions in the growth medium (Table 2), which have various roles in the physiology and biochemistry of plants in a saline environment, are absorbed by these plants. However, some previous studies on halophytes have shown that Na^+ , K^+ , and Cl^- accumulate to achieve osmotic adjustment by lowering solute potential and water potential in plant tissues (Yasseen & Abu-Al-Basal, 2008).

In this study, we have also focused our attention on the determination of a number of organic osmolytes like glucose, fructose, proline, glycine betaine, choline, choline-O-sulphate, and β -alanine betaine.

Organic solutes that are compatible with enzyme functions may thus play an important role in osmotic adjustment in the cytoplasmic compartment of plant cells. The compatible solutes synthesised by halophytes range from quaternary ammonium compounds through sulphonium analogues to proline and sugar alcohols; most are widely distributed through the orders of flowering plants, reflecting both phylogeny and functional needs (Flowers & Colmer, 2008).

Species-specific variations are seen not only in the nature of the organic osmolytes, but also in the seasonal pattern of osmolyte accumulation. The total amount of soluble carbohydrates (glucose and fructose) is highest in the summer in all 3 species (Table 3). Glucose levels decrease strongly in the autumn. A decrease is observed in the level of fructose in *L. iconicum*, while an increase is seen in *L. lilacinum*. However, it is found stable during the period of investigation in *L. anaticum*.

The investigated species show different abilities to accumulate proline (Table 3). In the autumn, a decrease is observed in the proline content in *L. anaticum* and *L. lilacinum*, while an increase is seen in *L. iconicum*. Studies for the last 5 decades have suggested that the accumulation of proline in tissues confers some salinity tolerance in different plant species, including halophytes (Orcutt & Nilsen, 2000; Kavi Kishor et al., 2005). However, the plants under investigation, from different locations, exhibit different abilities to accumulate proline, and the content of proline shows wide variations (Youssef et al., 2003). Such variations may be dependent on various factors, such as the environmental factors of habitats and energy constraints. In fact, the different views about the actual role of proline in the metabolism of plants under stress have generally been questioned (Yasseen & Abu-Al-Basal, 2008). However, glycine betaine accumulates in high concentrations in halophytes; according to many reports, it could be a main compatible osmolyte for the adaptability to saline environments (Rhodes & Hanson, 1993; Youssef et al., 2003).

Quaternary ammonium compounds such as glycine betaine and β -alanine betaine act as nontoxic (compatible) osmotica preferentially located in the cytoplasm and chloroplasts of plant cells; they also may act as enzyme protectants (Wyn Jones & Storey, 1981; Makela et al., 1998).

The Plumbaginaceae, a highly stress-tolerant family, contains species well adapted to a wide range of harsh environments, among which the *Limonium* species are found to accumulate a wide range of low-molecular-mass nitrogenous compounds including proline, pipercolic acid, β -alanine, γ -amino butyric acid, and quaternary ammonium compounds like β -alanine betaine and glycine betaine (Hanson et al., 1994; Bouchereau et al., 1999).

Hanson et al. (1994) reported the occurrence of several quaternary ammonium compounds in Plumbaginaceae: glycine betaine, β -alanine betaine, proline betaine, hydroxyproline betaine, and choline-O-sulphate. In particular, choline-O-sulphate and β -alanine betaine are found to accumulate in the Plumbaginaceae (Bouchereau et al., 1999). Rhodes and Hanson (1993) reported that *Limonium* species adapted to dry, sandy, or rocky soils tend to accumulate glycine betaine, whereas *Limonium* species colonising salt marshes tend to accumulate β -alanine betaine. Interestingly, substituting β -alanine betaine for glycine betaine in some species of Plumbaginaceae has not narrowed their adaptation, as many β -alanine betaine accumulators are found in dry saline environments as well as at the higher altitudes. β -Alanine betaine thus appears to be effective over a broader ecological spectrum than glycine betaine (Rhodes & Hanson, 1993). Similar results are found in the present study. Investigated *Limonium* species collected from the salt marshes of Lake Tuz accumulate different amounts of quaternary ammonium compounds, especially β -alanine betaine and choline-O-sulphate (Figure 4). The levels of β -alanine betaine and choline-O-sulphate change in parallel: the highest levels of β -alanine betaine and choline-O-sulphate are measured in the summer, and the lowest levels are measured in the autumn. In *L. iconicum*, the levels of β -alanine betaine and choline-O-sulphate are highest in the summer.

The plants under investigation from different times exhibit different abilities to accumulate proline, inorganic ions, soluble carbohydrates, and quaternary ammonium compounds. In the autumn an increase is observed in the concentration of Na^+ , Cl^- , and proline, while a decrease in the concentration of Ca^{2+} , Mg^{2+} , glucose, fructose, β -alanine betaine, and choline-O-sulphate is seen in *L. iconicum*. In *L. lilacinum* concentrations of Na^+ , Cl^- , proline, glucose, β -alanine betaine, and choline-O-sulphate decrease in the

autumn, but the concentration of fructose increases. In the autumn, a decrease is observed in the concentration of Ca^{2+} , Mg^{2+} , proline, glucose, β -alanine betaine, and choline-O-sulphate, while an increase is shown in the concentration of K^+ . These compounds might have been accumulated in the cytoplasm by the plants to contribute to the osmotic adjustment. These results prove each species has its own adaptation mechanism to survive in the saline soils.

Lake Tuz (Konya-Ankara-Aksaray) is surrounded by salty marshes. In the arid areas around this lake, there are halophytic vegetation zones with changing physiognomy and floristic compositions. Salt steppes and marshes of the Konya Basin, particularly those of the Lake Tuz area, show a very high proportion of plant endemism. Lake Tuz is also a rich region due to endemic halophytic plant species. Only these physiologically specialised species, characterised by the halophytes of the families Chenopodiaceae and Plumbaginaceae, can survive in saline soils. The saline conditions of Lake Tuz provide suitable areas for halophytic plants. The first studies of the terrestrial salty marshes of central Anatolia were related to the halophytic communities of Lake Tuz and the Konya plain (Aksoy & Hamzaoglu, 2006). Although there are some studies about the halophyte vegetation of region, there are not enough studies on the salinity tolerance mechanisms of these halophytes. The findings of this investigation are the first reported results for these 3 endemic species of *Limonium* located at Lake Tuz, Turkey. In addition to their economic importance, they could offer unique genetic pools to be used for gene technology programs.

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