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Mathematically Evaluation of Anatomical and Soil Properties of Some Limonium Mill. (Plumbaginaceae Juss) Taxa

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Abstract

In this study, the anatomical and soil properties of some Limonium taxa (*L. virgatum, L. graecum var. graecum, L. bellidifolium*) distributed on the salt marshes in the coastal Aegean of Turkey were studied and the data obtained were evaluated statistically. Anatomical sections were taken from root, stem and leave of plant samples belonging to the taxa. Measurements were made in these structures for nümerical analysis. Soil samples were taken from habitats where plant samples were taken. These soil samples were examined in terms of salt, conductivity and Na, P, K values. *L. graecum var. graecum* root, stem and leaf anatomy is different from other taxa. The number of salt pockets and stoma per unit area in the leaves of this taxon is higher than other taxa. The bulging epidermis cells, especially surrounding the salt pockets in the stem and leaves, are different from other taxa. This taxon lives in environments with the highest conductivity and sodium content and the lowest phosphorus level in terms of soil properties. *L. virgatum* has the lowest measurement results in terms of potassium, sodium, total Na+P+K and the number of stomata per unit area of leaves. It is important to determine the usefulness of Limonium species with beautiful looking flowers, which are frequently found in vacant lands on the shores of the Aegean Sea. The numerical data obtained from the laboratory studies were tried to be tested by comparing the features of the taxa. These comparisons are supported numerically by statistical analysis.

Keywords: Mathematically evaluation; Anatomy; Coastal Aegea; *Limonium*

Abbreviations: MS: Mean Square; NS: Not Significant.

Introduction

Plumbaginaceae Juss. is represented by 24 genera and 775 species in the world. Family members, which are generally distributed in arid and saline soils, are valuable as they are used as medicinal, ecological and ornamental plants [1]. Some cytological, morphological and anatomical studies have been performed on *Acantholimon Boiss ve Limonium Mill. genus* from *Plumbaginaceae family* [2-7]. *Plumbaginaceae taxa* are generally formed from halophytes plants. One of the most important features of this family is that it carries epidermal glands on its leaves and stems. These glands are called salt or lime pouch pocket [7]. In the *Limonium species*, salt pockets are located in the epidermis layer of the plant and directly throw the salt out of the cells [8]. Leaf stoma distribution rates may vary with habitat conditions [7]. *Limonium* is also known as “sea lavender”. The number of *Limonium species*, whose number is more than 350, increases with the new studies [9]. The IUCN hazard categories of taxa examined in this study are as follows; *L. graecum var. graecum* is in the

CR category, *L. virgatum and L. bellidifolium* are in the EN category. Salt stress is one of the most important abiotic stress factors for plants [10]. Halofit plants are capable of living at high salt concentrations [11-13]. In recent years, researchers have been working on identifying plant genotypes with high tolerance to salt stress and breeding new varieties that can live in areas with salinity and create economic products [14-16]. Plants under salt stress close their stomata, shrink leaf surface areas and try to prevent dehydration. However, the reduction of the leaf surface area reduces the photosynthesis per unit area and the growth and development of the plant decreases. Salinity creates oxidative stress in plant cells. Reactive Oxygen formed in plants under salt stress damages the cells [17]. Na + ions that accumulate in plant cells under salt stress and create toxic effects are balanced with K + ions taken into the cell [18,19]. These ions are taken from the soil with water and transported to the cells by vascular bundles. In this study, the anatomical structures and soil salinity properties of *Limonium virgatum Fourr., Limonium graecum var. graecum (Poir.) Kuntze, Limonium bellidifolium Dumort*. species belonging to *Limonium* genus were investigated. The results obtained were evaluated statistically and determined that which species were adapted to more saline environment, as a tolerance to salinity, measurements of cells in the vascular bundles and the degree of adaptation of cells in the leaf. Results are supported by tables and graphs.

Material and Methods

For anatomical investigations, the parts of the taxons were fixed in 70% ethyl alcohol. For microscopic observations, sections were taken from the parts of the plant and were stained using saffron and alcian blue dyes. Preparations prepared from these sections were examined using Leica DM3000 motorized microscope. The anatomical features were selected and measurements were made in these structures. The minimum, maximum, average and standard error values of the size of the cells belonging to the parts of each taxon were determined.

The soil samples were passed through a 2mm sieve and pre-selected measurements about salinity were made from the soils obtained. Conductivity measurement of samples was done with Thermo Scientific Orion 3 Star Conductivity meter. Phosphorus (P), Potassium (K) and Sodium (Na) analyzes were done with Optima 8000 ICP-OES in soil samples of plants.

The numerical datas of the anatomical and soil properties of the taxa examined were evaluated statistically by Pearson correlation analysis and Variance analysis. The taxa were coded as A-C. The anatomical and soil properties were coded as 1-8 (Table 1).

Code		A		B		C	
		L.virgatum		*L. graecum var. graecum*		*L. bellidifolium*	
		Min-Max	Ort.±ss	Min-Max	Ort.±ss	Min-Max	Ort.±ss
Diameter of root tracheal elements (µm)	1	14,71-64.71	42.13±16.21	10.59-64.71	37.03±20.41	17.65-88.24	53.67±23.53
Root vascular tissue / root cross section ratio (%)	2		53		71		57
Diameter of stem tracheal elements (µm)	3	13.24-35.29	24.56.±7.48	3.53-26.47	17.05±8.06	7.06-29.41	17.05±7.67
Stem scleranchyma tissue / stem diameter ratio (%)	4		20		17		15
Diameter of leaf tracheal elements (µm)	5	9.41- 32.35	20.38.±7.45	2.94-14.71	9.13±3.82	2.94-17.65	11.21±4.47
Leaf cuticle width (µm)	6	7.14-9.29	8.14±0.73	8.57-15.71	11.57±2.24	4.29-10.00	6.82±1.63
Unit area (1mm2) stoma number	7		46		68		52
Unit area (1mm2) salt pocket number	8		10		20		9

Table 1: Measurement results of some anatomical structures of examined Limonium taxa.

Results and Discussions

Anatomical Results

The root sections of the investigated taxa have a round shape. There is a peridermis protective tissue and a cortex layer under under the peridermis layer on the outermost part of the sections. Cortex layer covers a larger area in *L. virgatum and L. bellidifolium taxa*. Vascular bundles are located under the cortex layer. In L. graecum var. graecum, this layer continues to the core region. Also, parenchymatic cells are not observed in the core region. In the other two taxa, parenchymatic cells are located between the bundles. These cells can extend to the center of the section. They show a radial arrangement from the core region towards the outside of the section (Figure 1).

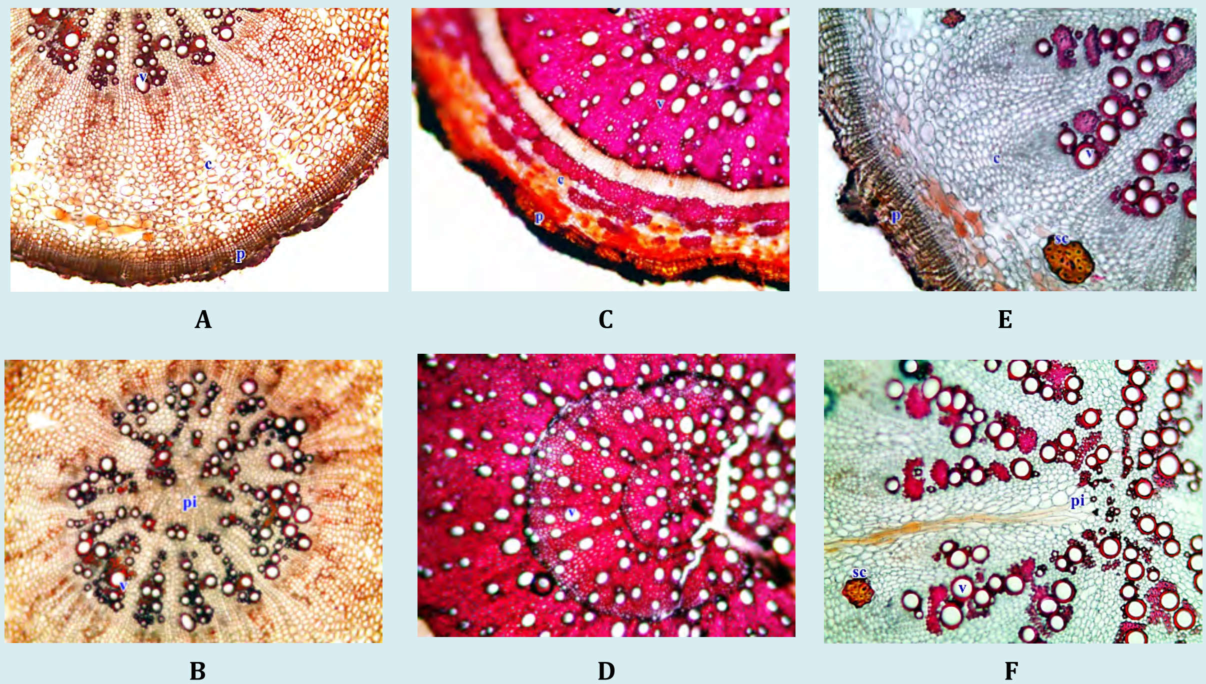
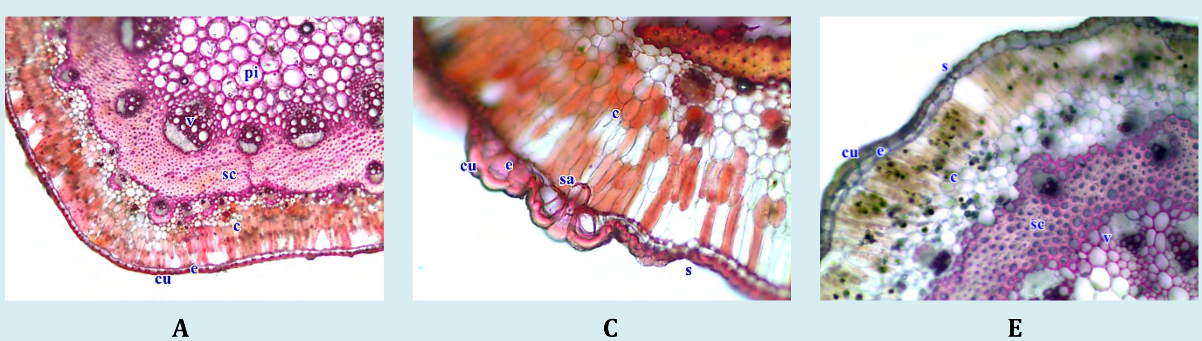
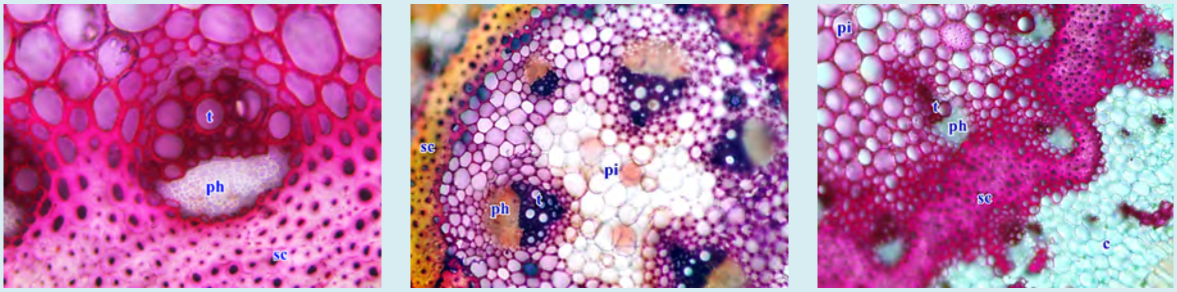


Figure 1: Root cross sections *L. virgatum (A,B) L. graecum var. graecum (C,D) L. bellidifolium (E,F) (p- Peridermis; co- Cortex; v- Vascular bundle; pi- Pith).*
(Microscope objective numbers: Ax10; Bx20; Cx10; Dx20; Ex10; Fx20).

The stem anatomical sections of the taxa have a round-oval shape. The epidermis cells include stomata and salt pockets. Especially in the *L. graecum var. graecum*, quite large and bulging epidermis cells are visible around the salt pockets. This taxon differs from other taxa in this aspect. *L. virgatum and L. bellidifolium* taxons have a narrow cortex layer under the epidermis, a row of vascular bundles in the interior of this layer, a layer of scleranchyma cells just below it, a second row of conduction bundles underneath this layer, and the parenchymatic cells in the center of the sections. *L. graecum var. graecum* contains the sclerancima cell layer under the cortex layer and a row of vascular bundles and parenchymatic cells under it (Figure 2).

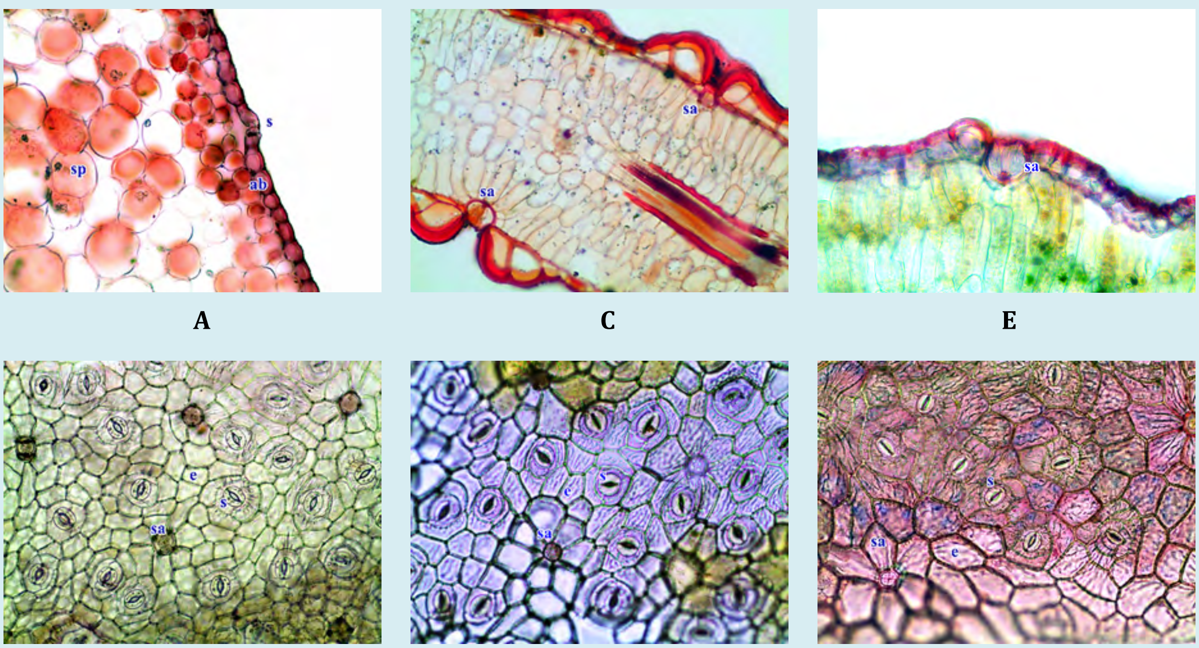




B D F

Figure 2: Stem cross sections- *L. virgatum (A,B) L. graecum var. graecum (C,D) L. bellidifolium (E,) F(cu- cuticle; e- Epidermis; sc- Sclerancymatic; c-Cortex; pi- Pith; s-stoma; sa-Salt pocket; v-Vasculer bundle; ph-Phloem; t- Trachea).*
(Microscope objective numbers: Ax10; Bx40; Cx20; Dx20; Ex10; Fx20).

Outside the adaxial and abaxial sides of the cross sections taken from the leaves of taxa, there is the cuticle layer, beneath it, the single row epidermis cells, and the cells forming the anisocytic stoma and salt pockets. The epidermis cells that make up the salt pockets of the *L. graecum var. graecum* are bulging outwards as in the stem. There are vascular bundles lined up at certain intervals in the mesophile layer (Figure 3).



A C E

B D F

Figure 3: leaf cross sections- *L.virgatum (A,B) L.graecum var. graecum (C,D) L. bellidifolium (E-F),(cu- cuticle; e- Epidermis; ad- Adaxial epidermis; pa- Palisade; sp-Sponge; ab- Abaxial epidermis; s-stoma; sa-Salt pocket; v-Vasculer bundle; ph-Phloem).*
(Microscope objective numbers: (Ax10; Bx20; Cx10; Dx20 ; Ex10; Fx20).

Results of Soil Analysis

The analysis results of the soil samples taken from the localities of the investigated taxa are shown in the Table 2. According to the table, the taxon that lives in the soil with the highest potassium concentration and total Na+P+K concentration is in *L. bellidifolium* with 6,0292 mg/g and 7,5425 and highest sodium concentration and lowest phosphorus concentration is in *L. graecum var. graecum* with 3,0675 mg/g and 0,0156 mg/g respectively. *L. graecum var. graecum* also lives in soils with the highest conductivity. The conductivity value of the soil in which this taxon lives is 2 times more than the conductivity value of the other two taxa. The conductivity values of the soil where the other two taxa live are close to each other. Due to the low amount of phosphorus in soils where *L. graecum var. graecum* lives, K/P ratio and Na/P ratio values are very high (Table 2).

	K (mg/g)	Na (mg/g)	P (mg/g)	Total Na +P+ K	K/Na ratio	K/P ratio	Na/P ratio	Conductivity (EC) (mS/cm)
L. virgatum	2.7505	0.4	0.6493	3.7589	7.66	4.2361	0.5531	7.42
L. graecum var. *graecum*	3.0036	3.1	0.0156	6.0867	0.98	192.54	196.63	14.86
L. bellidifolium	6.0292	1	0.8462	7.5425	5.55	8.72	1.33	7.14

Table 2: Soil analysis results of the examined *Limonium taxa*.

Results of Statistical Analysis

The anatomical measurements and soil features of the investigated taxa were shown in Tables 1 and 2. Significance of the differences between the investigated taxa was evaluated by analysis of variance (Regression Analysis) and Pearson correlation (Correlation). The statistical analysis of the results was given in Tables 3-8.

	1	2	3	4	5	6	7
1	0,565						
	0,618						
2	0,118	0,752					
	0,925	0,458					
3	0,231	0,672	0,993				
	0,851	0,531	0,050**				
4	0,050*	0,788	0,998	0,985			
	0,961	0,422	0,036*	0,109			
5	0,871	0,898	0,386	0,277	0,437		
	0,327	0,290	0,748	0,821	0,712		
6	0,520	0,999	0,787	0,711	0,820	0,873	
	0,652	0,034*	0,424	0,497	0,388	0,324	
7	0,781	0,957	0,528	0,427	0,575	0,987	0,940
	0,430	0,188	0,646	0,719	0,610	0,102	0,222

*Significant at the level of P< 0.05. ** Significant at the level of P< 0.01. Abbreviations: 1-8 : Codes of anatomical features.

Table 3: Pearson's correlation based on anatomical features.

	A	B	C	AS	BS
B	0,901				
	0,002**				
C	0,969	0,905			
	0,010**	0,002**			
AS	0,718	0,704	0,687		
	0,045*	0,051*	0,060		
BS	0,242	0,139	0,104	0,060	
	0,563	0,743	0,806	0,888	
CS	0,739	0,696	0,600	0,717	0,238
	0,036*	0,050*	0,116	0,045*	0,571

* Significant at the level of P< 0.05. ** Significant at the level of P< 0.01. Abbreviations: A-C : Codes of taxon, S: Total Soil features.

Table 4: Pearson's correlation based on anatomical and soil features of taxon.

	K	Na	P	NaPK	K/Na	k/P	Na/P
Na	0,327						
	0,788						
P	0,982	0,500					
	0,121	0,667					
Na P K	0,982	0,500	1,000				
	0,121	0,667	0,221				
K/Na	0,300	0,803	0,115	0,115			
	0,806	0,407	0,927	0,927			
K/P	0,169	0,987	0,352	0,352	0,889		
	0,892	0,104	0,771	0,771	0,302		
Na/P	0,140	0,890	0,049*	0,039*	0,987	0,952	
	0,910	0,302	0,969	0,969	0,105	0,198	
Cond.	0,189	0,866	0,010**	0,020*	0,993	0,936	0,999
	0,879	0,333	1,000	1,000	0,073	0,229	0,031*

Significant at the level of P< 0.05. ** Significant at the level of P<0.01.
Abbreviations: K : Potasyum, Na: Sodyum, P: Fosfor, Cond. : conductivity.
Table 5: Pearson's correlation based on Soil features.

Source	MS	F-value	Probability	Significance
A-B	1629.9	26	0.002	**
A-C	1882.9	91.77	0.41	**
B-C	3578.5	27	0.002	**

MS: Mean Square; *P<.05; **P<.01; A-C:
Codes of taxon; NS: Not Significant.
Table 6: Correlation between taxon (Analysis of Variance).

Source	MS	F-value	Probability	Significance
1-3	2.10	0.01	0.925	NS
1-4	2.10	0.01	0.925	NS
1-5	48.2	8.06	0.050	*
1-6	0.60	3.32	0.961	NS
1-7	41.3	0.37	0.652	NS
1-8	93.0	1.56	0.430	NS
2-3	101.1	1.31	0.458	NS
2-4	80.67	0.82	0.531	NS
2-5	111.0	1.64	0.422	NS
2-6	144.0	4.16	0.290	NS
2-7	178.1	345.6	0.034	*
2-8	163.5	10.79	0.188	NS
3-4	37.50	75.00	0.043	*

3-5	37.87	312.1	0.036	*
3-6	5.65	0.17	0.748	NS
3-7	23.52	1.62	0.424	NS
3-8	10.59	0.39	0.646	NS
4-5	2.589	33.33	0.109	NS
4-6	0.205	0.08	0.821	NS
4-7	1.347	1.02	0.497	NS
4-8	0.486	0.22	0.719	NS
5-6	13.13	0.24	0.712	NS
5-7	46.21	2.06	0.388	NS
5-8	22.72	0.49	0.610	NS
6-7	6.605	3.20	0.324	NS
6-8	8.445	38.2	0.102	NS
7-8	228.3	7.54	0.222	NS

MS: Mean Square; *P<.05; **P<.01; 1-2:
Codes of anatomical features; NS: Not Significant.
Table 7: Correlation between 8 anatomical features (Analysis of Variance).

Source	MS	F-value	Probability	Significance
K-Na	0.500	0.12	0.788	NS
K-P	4.100	27.0	0.120	NS
K-Con.	4.500	0.04	0.875	NS
Na-P	05.20	0.30	0.660	NS
Na/P-P	21.10	47.3	0.045	*
Na/P-NaPK	80.12	0.92	0.531	NS
Con.-Na/P	42.56	48.3	0.031	**
Con.-Na	41.30	0.37	0.652	NS
Con.-P	92.56	72.3	0.047	*
Con.-NaPK	0.248	0.14	0.771	NS
P-Na	0.500	0.33	0.667	NS
P-NaK	37.87	32.1	0.036	*

MS: Mean Square; *P<.05; **P<.01; 1-2:
Codes of anatomical features; NS: Not Significant.
Table 8: Correlation between 8 soil features (Analysis of Variance).

When the anatomical features were evaluated with both statistical analysis methods, there were differences between the characters 1-5, 2-7, 3-4 and 3-5 at the significance level of 0.01P and 0.05P, and the results are given in Tables 1,3,7.

In Table 3, pearson correlation test was applied between the anatomical features and soil features of the taxa examined and significant differences were found between A-B, B-C, A-C taxa and between A-AS, A-CS, B-AS, B-CS and AS-CS features. Table 4 shows that all taxa are related to each other as a result of variance analysis.

Pearson correlation test was applied between the soil properties of taxa examined in Table 5 and significant differences were found between P-Na / P, P-conductivity, NaPK-Na / P, NaPK-conductivity and Na / P-conductivity characters. When the results of variance analysis of soil properties of all taxa are evaluated in Table 6, Na / P-P, conductivity-Na / P, conductivity-P, P-Na / K characters appear to be related.

In this study, anatomical and soil properties of three *Limonium taxa* that spread in coastal Aegean were examined and the results were evaluated statistically. In terms of root, stem and leaf anatomical features, *L. graecum var. graecum* has different features than the other two taxa. This taxon differs from the other two taxa with xylem elements filling the center at the root, bulging epidermis cells in the stem and leaf epidermis, the thickness of the cuticle layer, the excess of the number of salt pockets and stomata per unit area in the leaf. This taxon also has the highest conductivity (total salt ratio) with 14.86mS / cm. Halophytes plants expand the leaf surface, thicken its cuticle, increase the number of stomata and expel the excess salt dissolved in the water by gutation, to cope with soil salinity [20]. *L. graecum* var. graecum is the type that prefers the most saline soils with conductivity value of 14.86mS / cm. Although this taxon has many base leaves, it does not carry leaves on the stem. For this reason, it complements the deficiency of the leaf with many salt pockets and stoma in his stem. This situation was also detected in anatomical studies. The most prominent salt pockets among the taxa examined were observed in the stem and base leaves of this taxon. In fact, the epidermis cells next to these salt pockets differ in shape and size from other epidermis cells with their bulging structures. It can be said that this taxon copes with the soil salinity in the areas where it lives with these anatomical features. This species also has less spread than other taxa. Due to the high salinity of the species in the habitat, the number of plant species to which it competes decreases. In this case, it can be said that taxon adapts to salty soils and turns soil salinity into an advantage.

Conclusion

In this study, the data of the anatomical and soil properties of the taxa examined were evaluated statistically by Pearson correlation analysis and Variance analysis. Both statistical analysis data yielded consistent results.

It has been observed that the results of different statistical tests and graphics belong to the investigated taxa have mostly similar results and provide parallelism. Based on this, we can say that the results obtained from the study are reliable. According to the statistical evaluation results; According to K and P analyzed for Na, which is one of the soil chemical properties; It has significant differences with the soil properties of plants and it is seen to be statistically significant. Likewise, it is seen that this element has a strong correlation with the anatomical features of plants with P values at 0.05 and 0.01 levels (Tables 5,8). We cannot say this statistical evaluation we determined for Na for K and P. Because if we compare the results obtained from statistical studies; No statistically significant correlation was found between K and P elements and the anatomical and soil properties of *Limonium taxa*, which are the subject of our study (Tables 5 & 8). The reason for this situation may be that K and P are mobile mineral elements. There is information in the literature that K is a more mobile element than P. The statistical results of K in this study support this situation and when we look at the statistical results of P and K, it is clearly seen that the anatomical and soil properties and the results of K have less correlation than the results of P [21].

It is noteworthy that there is a directly proportional relationship between Na amounts of taxa and conductivity values. *L. graceum taxon*, which is distributed in the soils with the highest Na value, also shows distribution in the soils with the highest conductivity value. *L. virgatum*, which has the lowest Na value soils, is partly distributed in soils with low conductivity.

Statistical results show that the anatomical features of taxa are related to soil salinity. The anatomical results we photographed under the microscope of the plants also show the existence of this relationship. Taxa that distributed on soils with higher total salinity values confirm these results with their different anatomical structures. Especially, this connection is more noticeable in the anatomical features of the leaf.

It was observed that the anatomical characteristics of the studied taxa were related to the total salinity rates of the soils they spread. While *L. graceum* has the largest leaf cuticle, it also has the highest conductivity value. This result confirms our observations. Salty plants cope with soil salinity with large area cuticles and numerous stomata. *Limonium graecum* prefers the most salty soils. Although this type has many base leaves, it does not have leaves on the stem. For this reason, it complements the lack of leaves with a large number of salt pockets and stomata it carries on its body. Among the taxa studied, the most prominent salt pockets were observed in the stem and base leaves of this species. It can be said that this taxon can cope with soil salinity in the areas where it lives with these anatomical features. Due to the high salinity in the habitat of the species, the number of plant species with which it competes is decreasing. In this case, it can be said that the taxon adapts to salty soils and transforms soil salinity into an advantage. When the data obtained as a result of the study were evaluated, the existence of a relationship between the anatomical features of the taxa and soil salinity was concretized by statistical studies, and it was observed that it was parallel to laboratory studies.

Increasing drought and soil salinity negatively affect the vegetation cover, and this situation makes it important to identify these economically important plant species that are the subject of our study and can adapt to these negative environments.

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