



Ecology of *Trifolium elazizense*, an Endemic Alpine Meadow Species from Türkiye

Mustafa KESKİN^{1*} , Veysel SONAY² , İbrahim Ertuğrul YALÇIN³ ,
Volkan ALTAY⁴ , Münir ÖZTÜRK⁵ 

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Corresponding Author e-mail:
trifolium@hotmail.com

Institution: ¹ Marmara University
² Harran University
³ Bahçeşehir University
⁴ Hatay Mustafa Kemal University
⁵ Ege University

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ABSTRACT

This study presents some ecological characteristics of *Trifolium elazizense*, a local endemic alpine meadow plant species in the East Anatolian region of Türkiye. Elemental analyses were carried out in plant and soil. Soil-plant relationships were evaluated. The results obtained from all plant parts of *Trifolium elazizense* (root, stem and leaves) showed that the elemental concentrations of boron, calcium, copper, potassium, manganese, lead and zinc were within the limit values; cadmium, iron and magnesium were above the limit values. The general concentration of all elements in the soil samples is generally within acceptable limits.

Türkiye'den Alpinik Çayırlarda Yetişen Endemik *Trifolium elazizense* Türünün Ekolojisi

ÖZET

Bu çalışma, Türkiye'nin Doğu Anadolu bölgesinde lokal endemik, alpin çayır bitkisi olan *Trifolium elazizense* türünün bazı ekolojik özelliklerini sunmaktadır. Toprakta ve bitkide element analizleri yapılmıştır. Toprak-bitki ilişkileri değerlendirilmiştir. *Trifolium elazizense* türünün tüm bitki kısımlarından elde edilen sonuçlar (kök, gövde ve yapraklar) element konsantrasyonlarının bor, kalsiyum, bakır, potasyum, manganez, kurşun ve çinko değerleri sınır değerlerinin; kadmiyum, demir ve magnezyum ise sınır değerlerinin üzerindeydi. Toprak örneklerindeki tüm elementlerin genel konsantrasyonu genel olarak kabul edilebilir sınırlar içindedir.

1. INTRODUCTION

The diversity of plant species in an ecosystem plays an important role in its stability as well as productivity and contributes significantly to the maintenance of ecological functions, especially the continuity of nutrient/water cycles together with the continuation of vital activities [1-9]. For this reason, examining the plant diversity present in different ecosystems and elucidating the ecological relationships with their habitats

greatly contributes to the understanding of conservation biology of sensitive plant taxa, particularly the relict, rare and endemic plants found in such ecosystems [10-13].

The variability of microclimatic conditions at different altitudes of mountain ecosystems too is reflected in the diversity of plants, especially endemics, found in such habitats [14]. The reason being in parallel with changes in the altitude factor of mountain ecosystems; these cause significant changes in the existing ecological conditions, especially in climate, plant ecophysiology and soil dynamics [15]. These ecosystems; especially alpine and subalpine ones; play an important role through their different ecological functions [16]. Mountain ecosystems are among the ecological environments characterizing isolated environments, including special areas where sensitive plants such as relict, rare and endemic plants grow. They show different plant diversity [17].

Knowledge about the habitat preferences of endemic plants distributed in restricted areas facilitates the understanding of plant-soil relationships in ecological terms [13]. Türkiye has an important position in terms of eco-genetic resources because of its location at the intersection of biodiversity gene centers of the Middle East and the Mediterranean [13, 18]. The country has a high proportion of endemic plants (nearly 31.12 % of the total flora). The reason is country's diverse geomorphological, topographical and climatic features as well as a wide range of habitats [19-21]. The list of endemic plant taxa is also given in the floristic studies conducted by some researchers [22-26]. The researchers have conducted studies on the ecological characteristics of some endemic, rare and relict woody, shrub and herbaceous plants of ecologically sensitive importance in Türkiye [4, 13, 27-33].

The genus *Trifolium L.* is one of the largest genera in the family Fabaceae, with approximately 300 species distributed worldwide [34]. This genus is one of the most economically important genera due to its widespread use as fodder and green manure and its ability to fix nitrogen through its roots [35, 36]. It is also recorded as one of the genera with the highest species richness in the Mediterranean basin [37]. The genus is represented by 105 species in Türkiye [38, 39]. Many systematic and morphological studies have been conducted on this genus in Türkiye during the last 2 decades [38-48].

Species richness and composition of flowering plants in grasslands is mainly determined by abiotic factors such as topographic and climatic parameters [3, 49]. Among these factors, the effect of microclimate is regarded as the most important one [49]. The determinants of plant species richness and composition in grasslands are often interrelated and have common effects on plant diversity [49]. In view of this situation there is a general lack of information on the ecology of endemic plant species found in alpine meadows. No detailed ecological study has been conducted on the current status of the naturally distributed endemic *Trifolium elazizense M. Keskin, Sonay and Balos*. The present study is the first of its kind undertaken to understand the ecological characteristics of *T. elazizense*.

2. MATERIALS and METHODS

2.1. Studied species

Trifolium elazizense (Figure 1) is a locally endemic plant species, introduced to the scientific world in 2023 [38]. This endemic species is found in alpine meadows on northern slopes, at altitudes between 2100-2300 m in the East Anatolian region of Türkiye.

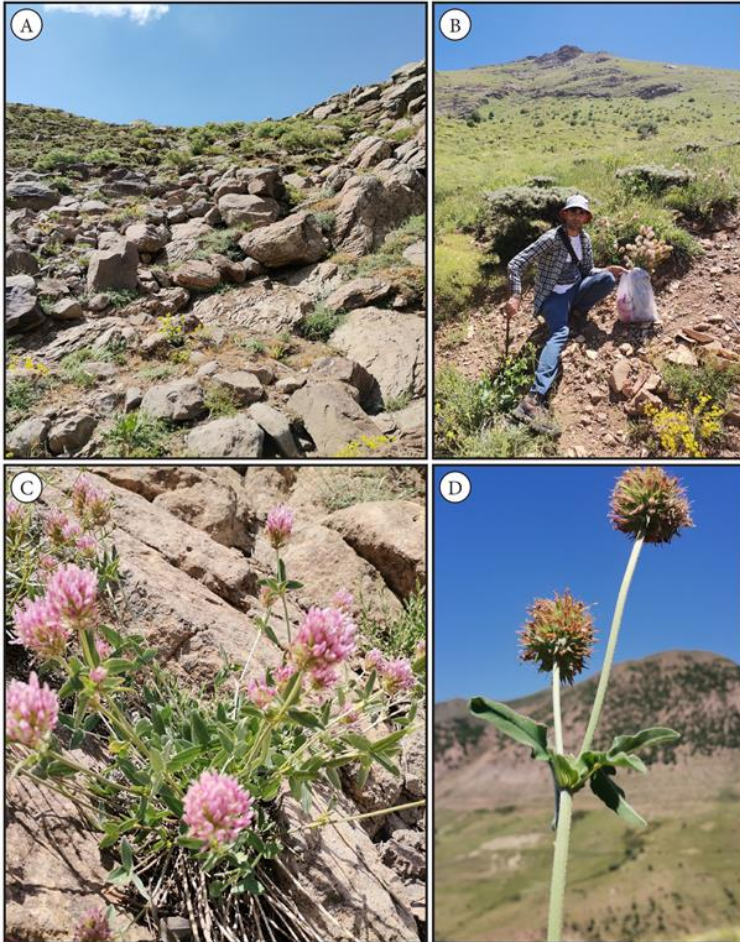


Figure 1: Some photos of the endemic *Trifolium elazizense* [A The natural habitat of the plant; B Collection of plant samples, researcher V. Sonay; C General view of the plant; D Fruit branches of the plant]

2.2. Study sites

The plant and soil samples were collected from six different sites in the alpine vegetation layer of the East Anatolian region of Türkiye, namely Elazığ-Karakoçan: Sarıcan district, Kejikan highland, northern slopes (2100-2300 m) for the endemic *T. elazizense*.

2.3. Data analysis

Plant samples were separated into leaves, stems and roots. These were individually wrapped in coarse filter paper and labelled accordingly. To inhibit mold growth, all plant materials were oven dried at 80°C for 48 hours at the laboratory to achieve a constant dry weight. Soil samples were placed in glass Petri dishes and

oven dried at 80°C for 48 hours. After drying, the samples were sieved through a 2 mm pore diameter steel mesh to remove large particles and prepared for weighing. Both plant and soil samples were accurately weighed in the range of 0.2000-0.2250 grams using an analytical balance (Precisa XB 220A SCS) and transferred to Teflon vessels, designed for microwave digestion system (Berghof - MSW2) [13, 33, 50, 51].

The plant samples were digested with 8 mL of 65 percent nitric acid (HNO₃). In comparison, the soil samples required a more complex digestion protocol consisting of 6 mL of 65 percent HNO₃, 3 mL of 37 percent hydrochloric acid (HCl) and 2 mL of 48 percent hydrofluoric acid (HF). All chemical reagents used were purchased from Merck. After digestion, samples were filtered through Whatman blue band filter paper into sterile 50 mL Falcon tubes using ultrapure water [50]. Following these preparatory steps, elemental analyses for boron (B), calcium (Ca), cadmium (Cd), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), lead (Pb) and zinc (Zn) were performed using inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer - Optima 7000DV) [13, 33, 50, 51].

2.4. Statistical analysis

All elemental concentration calculations were performed using the dry weight of soils, roots, stems and leaves. Statistical analysis for Pearson correlation was performed using IBM SPSS Statistics 25 software. The levels of statistical significance were indicated as $**p < 0.01$ and $*p < 0.05$, and a two-tailed test was used for the analysis [33, 50, 51].

3. RESULTS

The results of the elemental analysis of the plant and soil samples of *T. elazizense* are presented in Table 1.

Table 1. Elemental analysis of the plant and soil samples of *Trifolium elazizense*

Elements	Soil samples (mg kg ⁻¹)	Plant samples		
		Root (mg kg ⁻¹)	Stem (mg kg ⁻¹)	Leaves (mg kg ⁻¹)
B	54,738-62,711	15,575-17,134	7,634-8,401	11,155-12,266
Ca	8254,402-9449,931	5138,747-5609,526	2521,858-2752,914	3685,738-4023,415
Cd	0,944-1,234	1,564-1,755	0,793-0,874	1,116-1,229
Cu	43,727-50,230	10,588-11,501	5,208-5,686	7,583-8,245
Fe	5098,975-5837,545	986,513-1076,810	484,133-528,479	707,554-772,339
K	10591,701-12125,766	4196,073-4580,420	2059,243-2247,856	3009,613-3285,307
Mg	2923,619-3347,107	2213,199-2415,915	1086,165-1185,630	1587,406-1732,805
Mn	543,082-621,798	200,583-218,899	98,450-107,441	143,846-156,974
Pb	17,112-19,739	5,700-6,159	2,825-3,031	4,088-4,394
Zn	133,904-153,435	42,077-45,882	20,655-22,527	30,174-32,878

In all plant parts of *T. elazizense* (root, shoot and leaf) the element concentrations of B, Ca, Cu, K, Mn, Pb and Zn were determined to lie within the limit values whereas, Cd, Fe and Mg were above the limit values. The general concentration of all elements in the soil samples is generally within the acceptable limits (Tables 1 and 2).

Table 2. Limit values of elements for plant and soil samples [49, 51, 52]

Elements	Soil samples (min.-max.) (mg kg ⁻¹)	Plant samples (min.-max.) (mg kg ⁻¹)
B	20-200	3-90
Ca	7000-15000	3000-30000
Cd	0.06-1.1	0.05-0.5
Cu	25-75	5-30
Fe	5000-50000	50-250
K	5000-25000	1000-50000
Mg	300-8400	100-1000
Mn	10-9000	30-300
Pb	10-40	5-30
Zn	3-300	20-150

Following an evaluation of the concentrations of all elements we found these to be significantly higher in roots than in other parts of the plant (root > leaves > stem). The highest concentrations in plant parts were recorded for Ca; all elements with the highest concentrations generally followed the order Ca > K > Mg > Fe > Mn > Zn > B > Cu > Pb > Cd. Considering the existing concentrations of these elements in the soil samples, the following order was noted K > Ca > Fe > Mg > Mn > Zn > B > Cu > Pb > Cd. The lowest concentration was recorded for Cd in all parts of the plant, as well as in the soil samples. The results obtained in this study revealed that this plant species is able to extract all elements necessary for its growth from the soil where it grows. Similar findings have been reported by Lopes et al. [55] under a similar situation.

An examination of the Pearson correlation coefficients was done between the concentrations of elements in all plant parts (leaves, stem and root) of *T. elazizense* and soil samples taken from its habitat (Table 3), no significant positive or negative correlation was found between Cd and the other elements investigated in this study. On the contrary, a high positive correlation (>0.99, >0.83) was found between all the elements B, Ca, Cu, Fe, K, Mg, Mn, Pb and Zn.

Table 3. Pearson's correlation matrix (R) between the elements determined in all the parts of *T. elazizense* and its soil samples

<i>Correlation Matrix (R)</i>									
<i>Pearson Correlation</i>	Ca	Cd	Cu	Fe	K	Mg	Mn	Pb	Zn
<i>B</i>	.907**	-.085	.875**	.898**	.987**	.894**	.898**	.919**	.951**
<i>Ca</i>		.199	.849**	.840**	.947**	.986**	.872**	.965**	.962**
<i>Cd</i>			-.112	-.140	-.009	.358	-.029	-.055	-.067
<i>Cu</i>				.881**	.904**	.882**	.826**	.958**	.939**
<i>Fe</i>					.921**	.868**	.914**	.954**	.917**
<i>K</i>						.927**	.935**	.935**	.978**
<i>Mg</i>							.909**	.907**	.903**
<i>Mn</i>								.984**	.969**
<i>Pb</i>									.886**

**Correlation is significant at the level of 0.01 (2-tailed).

*Correlation is significant at the level of 0.05 (2-tailed).

Although Pb, like Cd, is a toxic heavy metal for plants, it seems to have a balanced interaction with other macro- and micro-elements and essential heavy metals for plant growth in our plant. However, this balanced interaction does not apply to Cd. This can be explained by the presence of levels in the plant parts (Tables

1 and 2), which indicate that Pb is below toxic thresholds, whereas Cd exceeds toxic levels. No disturbance in the photosynthetic reactions of this endemic plant were found, no delay in flowering and ripening times, no damaged tissues such as necrosis and chlorosis in the leaves. Lack of deficiency of macro and microelements shows that the plant can easily tolerate the toxic metal Cd as a reflection of its ecological adaptation.

4. CONCLUSION

Understanding endemic plants and their habitats from an ecological perspective contributes to their conservation. The results presented here include ecological findings on the local endemic alpine meadow species of Türkiye, *T. elazizense*. This study preliminarily contributes towards elucidation of ecological requirements as well as habitat preferences of *T. elazizense*.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the author.

Author's Contribution

All authors contributed in almost equal proportions.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

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Declaration of research and publication ethics

The authors of the paper declare that we followed the scientific, ethical and citation rules of Environmental Toxicology and Ecology in all processes of the paper and that we did not make any falsification of the data collected. Furthermore, we declare that ETOXEC and its Editorial Board are not responsible for any ethical violations that may have occurred and that this study has not been evaluated in any other academic publication environment than ETOXEC.

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