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Investigation of feed value and heavy metal content of some *Trıfolium* L. (Fabaceae) species in high mountain sections of Çamlıhemşin (Rize, Turkiye)

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Abstract: This study investigates the forage productivity, feed value (caloric), and heavy metal content of selected species of the genus *Trifolium* (Fabaceae) distributed in the high mountain pastures of Çamlıhemşin (Rize, Turkiye). Fieldwork was conducted during the flowering period of 2013–2014 in the Verçenik, Gito, Kavron, Çeymakçur, Elevit, Incesu and Çat plateaus. Five commonly occurring species (*Trifolium pratense*, *T. repens*, *T. ambiguum*, *T. spadiceum*, and *T. canescens*) were collected, herbarized, and analyzed. Productivity and hygroscopic moisture content were determined by biomass sampling, while caloric values were measured with a bomb calorimeter. Heavy metal concentrations (Al, Cd, Pb, Ni, Cr, Cu, Fe, Zn, Mn, Co) were quantified using ICP-OES after wet digestion. Results indicated that *T. canescens* and *T. ambiguum* exhibited the highest productivity and moisture content, whereas *T. spadiceum* showed the lowest. Caloric values ranged between 4369–4714 J/g in 2013 and 17729–20209 J/g in 2014, with *T. pratense* and *T. canescens* recording the highest values. Significant interspecific and interannual differences were observed in heavy metal concentrations, with *T. pratense* generally accumulating higher levels of Al, Fe, Cu, Cr, Ni, Pb, and Co, while *T. repens* showed higher Mn and Zn content. These findings confirm that *Trifolium* species are valuable forage resources in the region, though variability in heavy metal accumulation highlights the importance of monitoring ecological and anthropogenic impacts on high-mountain pastures.
Keywords: *biomass sampling, caloric value, forage resources, ICP-OES, variability*

INTRODUCTION

Forage crops are the cornerstone of ruminant nutrition, particularly in mountainous regions where grazing-based livestock systems prevail. In Turkiye, high-altitude pastures are ecologically rich but increasingly fragile due to both natural limitations and anthropogenic pressure [Greveniotis et al., 2025]. Among forage plants, species belonging to the genus *Trifolium* L. (Fabaceae) are extensively utilized because of their high protein content, favorable palatability, and capacity to improve soil fertility via nitrogen fixation [Acar, Ayan, 2012].

Leguminous forages are known to absorb and translocate heavy metals differently depending on species, soil chemistry, and environmental conditions. Elevated concentrations of trace metals can impair plant physiological functions such as photosynthesis and nutrient uptake, and may ultimately lead to toxicity in livestock through biomagnification [Sharma, Agrawal, 2005]. Despite the ecological and agricultural importance of *Trifolium* species, integrated studies evaluating both forage quality (e.g., biomass and energy content) and environmental safety (e.g., heavy metal levels) in Turkish alpine ecosystems remain scarce.

Trifolium species such as *T. pratense* L., *T. repens* L., and *T. ambiguum* M. Bieb. are commonly found in alpine and subalpine zones of northeastern Turkiye, including the Rize province, which is known for its diverse flora and steep agroecological gradients [Atamov, 2021]. These species contribute significantly to the local fodder economy through both biomass yield and nutritive value. However, environmental quality – particularly soil contamination from heavy metals – poses a growing threat to forage safety and productivity.

Heavy metals such as cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) can accumulate in plant tissues even at low soil concentrations, potentially affecting plant physiology and entering the food chain through grazing animals [Karahan, 2023; Hasan et al., 2025]. This is of particular concern in montane ecosystems, where limited soil depth, high rainfall, and proximity to touristic or pastoral activity may exacerbate metal mobility and uptake [Angon, 2020].

Despite their importance, few studies have simultaneously examined forage productivity, caloric energy content, and heavy metal accumulation across

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multiple *Trifolium* species within Turkish alpine pastures. Most existing work focuses on either agronomic yield or environmental contaminants in isolation.

This study offers a novel, multi-parameter evaluation of five *Trifolium* species (*T. pratense*, *T. repens*, *T. ambiguum*, *T. spadiceum*, *T. canescens*) distributed across seven high-altitude plateaus in Çamlıhemşin (Rize, Turkiye). It uniquely combines field-based biomass sampling, caloric energy analysis, and ICP-OES quantification of ten heavy metals to provide an integrated understanding of the forage potential and environmental sensitivity of these native species. Such a holistic approach has not previously been applied to mountain pasture legumes in the region.

MATERIAL AND METHODS

This study was carried out with the aim of determining the species belonging to the genus *Trifolium* L., which are distributed in the high mountain areas within the borders of Çamlıhemşin province, in the flowering phenophase in 2013-2014. Field studies were carried out in the Verçenik, Gito, Kavron, Çeymakçur, Elevit and Palovit plateaus (Fig. 1).

The regions where the plant samples collected from the research area, the dates and coordinates of the samples are given in Table 1. Plant samples of each species are herbarized and stored in Recep Tayyip Erdoğan University Herbarium (RTEUB).

They are widely distributed in the areas used as pastures of the region, especially in the Kavron, Gito, Elevit plateaus, and these are species with dense populations.

Wet Combustion Method and Elemental Analysis. The ground plant samples of about 0.3 g were weighed and placed in Teflon containers, and 5 ml of 65% HNO_3 (Nitric acid) and 3 ml of 30% H_2O_2 were added. After closing the lid of the Teflon container, shaking it and waiting for 20 minutes, it was placed in the microwave (Berghof Speedwave, Germany) and the wet burning process was carried out using appropriate programs for plant and soil samples. The samples that had finished the wet burning process in the microwave were then transferred into the liquid medium.

The solutions in Teflon containers were filtered with filter papers and the final volume was completed with pure water to 50 ml (EPA Method 3052). The heavy

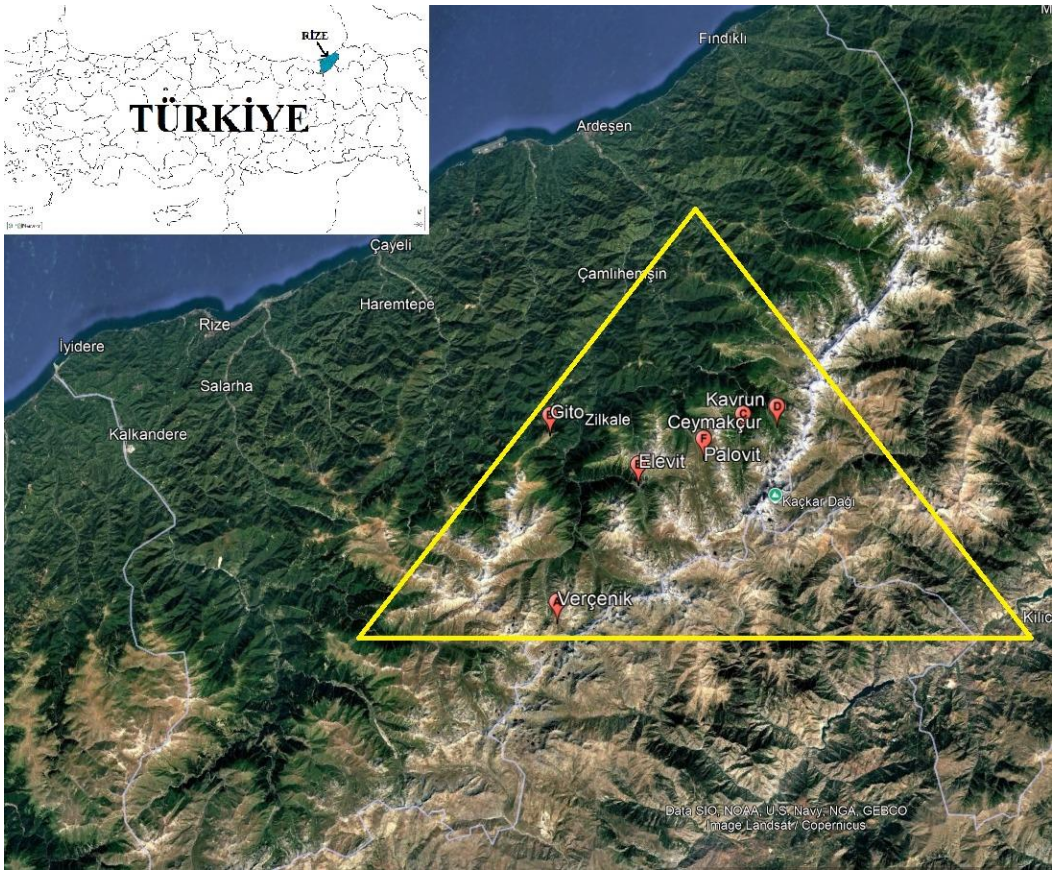


Figure 1. Image of the research area.

Table 1. Areas, dates and coordinates of the field studies.

Area	Departure dates	Height (m)	Species name	Coordinates
June-July, 2013				
Verçenik	27.06.2013	2510	*Trifolium pratense*	X:656708; Y:4515439
Gito	21.06.2013	1977	*Trifolium pratense*	X:660829; Y:4530098
Kavron	08.07.2013	2239	*Trifolium repens*	X: 679540; Y:4529258
Elevit	26.10.2013	1850	*Trifolium ambiguum*	X:669123; Y:4525149
Çat	07.06.2013	1277	*Trifolium spadiceum*	X: 663398; Y:4525313
İncesu	06.07.2013	1000	*Trifolium canescens*	X:0648712; Y:4527025
Ceymakçur	28.07.2013	2000	*Trifolium ambiguum*	X: 683346; Y: 4533168
June-July, 2014				
Verçenik	25.06.2014	2100	*Trifolium pratense*	X:659797; Y:4517908
Gito	21.06.2014	1855	*Trifolium pratense*	X:680123; Y:4555674
Kavron	19.07.2014	2012	*Trifolium repens*	X:679261; Y:4527878
Elevit	18.07.2014	1965	*Trifolium spadiceum*	X:669970; Y4524595
Çat	25.06.2014	1280	*Trifolium ambiguum*	X:663673; Y: 4525219
Ceymakçur	20..07.2014	1974	*Trifolium canescens*	X:682972; Y:4533283

metal values of the samples transferred to the liquid medium were read in ppm on the Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)? (PerkinElmer, Optima 7000 DV Model).

Heavy metal concentrations (mg/L; ppm) obtained from the ICP-OES device were statistically evaluated using the Statistical Package for the Social Sciences (SPSS 17.0). Differences in element concentrations among the common *Trifolium* L. species were examined by applying a one-way analysis of variance (One-Way ANOVA) following the procedures of [Fisher, 1925] and [Snedecor, Cochran, 1989]. The analyses were performed both between species and across sampling years. When the ANOVA indicated significant effects, Tukey's Honestly Significant Difference (HSD) post hoc test was applied to identify pairwise differences among species, based on the multiple comparison approach described by [Tukey, 1949; Hsu, 1996].

RESULTS AND DISCUSSION

According to the flora studies conducted in the research areas, the species of *T. arvense* L., *T. repens* L., *T. canescens* Willd, *T. pratense* L., *T. spadiceum* L., *T. nigrescens* Viv., *T. ambiguum* M. Bieb., *T. aureum* Pollich, *T. campestre* Schreb. are widespread. Five of these species are commonly found in the research region that include *T. pratense* L. (Meadow clover), *T. repens* L. var. *repens* (White clover), *T. ambiguum* M. Bieb. (Pik's ear), *T. spadiceum* L. (Meadow mulberry), *T. canescens* Willd (Yellow clover) (Fig. 2).

To determine the aboveground herb productivity of the investigated species, aboveground plant mass was taken from a 1 m^2 sample area and dried until it reached a constant weight. As a result of the applied drying process, the average productivity of these species and the average hygroscopic moisture rate were determined by finding the difference between fresh and dry plant masses (Tab. 2).

As can be seen from the table 2 data for 2013, *T. pratense*, *T. canescens* and *T. ambiguum* are the species with the highest productivity, which varies between 125-105 gr/m^2. The productivity of *T. repens* var. *repens* and *T. spadiceum* species is lower, with variations between 85-32 gr/m^2 (Tab. 2).

The amount of hygroscopic moisture in these species was determined. Productivity was found out to be higher in *T. canescens* (317 gr/m^2), *T. ambiguum* (301 gr/m^2) and *T. pratense* (287 gr/m^2) species, and ranged in *T. repens* var. *repens* and *T. spadiceum* between 175-80 gr/m^2. We believe that this difference is related to the habitat in which the plants live, as well as to the ecological characteristics of the plants and the aspect and direction of the slope (Tab. 2).

According to 2014 data, *T. pratense*, *T. canescens* and *T. ambiguum* are the species with the highest productivity. The productivity of these species varies between 102-130 gr/m^2. The productivity of *T. repens* var. *repens* and *T. spadiceum* species is lower. It has been determined that it varies between 38-90 gr/m^2.

The amount of hygroscopic moisture was determined and it was higher in *T. canescens* (294 gr/m^2), *T. ambiguum* (308 gr/m^2) and *T. pratense* (260 gr/m2), and ranged between 63-162 gr/m^2 in *T. repens* var. *repens* and *T. spadiceum*. It was concluded that these results

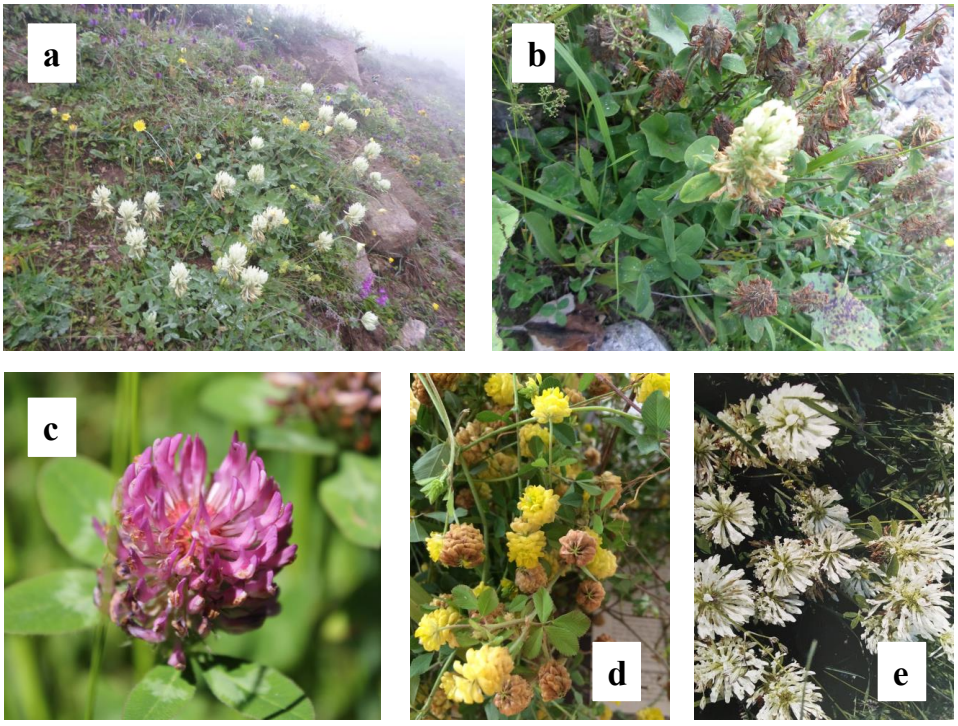


Figure 2. Images of species belonging to the genus *Trifolium* from the research area: a) *T. ambigium* (Verçenik Plateau), b) *T. canescens* L. (Kavron Plateau), c) *T. pratense* L. (Komati Plateau), d) *T. repens* Willd. (İncesu Plateau), e) *T. sapadiceum* (Elevit Plateau).

depend on the environmental conditions and habitat characteristics in which the species are distributed.

Caloricity of Trifolium species in the research area. In order to determine the amount of calories in collected and dried plant samples, the amount of energy in the five plant samples of the *Trifolium* was determined as cal/g. The calorie content of these *Trifolium* species is given in the table below (Tab. 3).

The caloric values of the five *Trifolium* species were determined for the 2013 and 2014 sampling periods, and clear interspecific as well as interannual variations were observed. In 2013, the energy content ranged from 4369 to 4714 J/g, with *T. canescens* having the highest caloric value (4714 J/g), followed by *T. repens* (4547 J/g). The lowest energy content was recorded in *T. spadiceum* (4369 J/g). The caloric values of *T. pratense* (4412

Table 2. Fresh and dry weights and hygroscopic humidity ratio of species belonging to the genus *Trifolium* L. (June-July, 2013-2014).

Species	Wet Weight (gr/m^2)	Dry Weight (gr/m^2)	Hygroscopic Humidity Rate (gr/m^2)
June-July 2013			
Trifolium pratense	412	125	287
Trifolium repens var. *repens*	260	85	175
Trifolium ambiguum	406	105	301
Trifolium spadiceum	112	32	80
Trifolium canescens	436	119	317
June-July 2014			
Trifolium pratense	390	130	260
Trifolium repens var. *repens*	252	90	162
Trifolium ambiguum	422	114	308
Trifolium spadiceum	101	38	63
Trifolium canescens	396	102	294

Table 3. Calorimetric results of species belonging to the genus *Trifolium* L. on a yearly basis (2013-2014).

Species	Sample weight (g)	Amount of energy (joule/g)
June-July 2013		
Trifolium ambigium	1.068	4408
Trifolium repens var. *repens*	1.022	4547
Trifolium canescens	1.011	4714
Trifolium pratense	1.009	4412
Trifolium spadiceum	1.009	4369
June-July 2014		
Trifolium ambigium	1.053	17729
Trifolium repens	1.005	19301
Trifolium canescens	1.017	19395
Trifolium pratense	1.025	20209
Trifolium spadiceum	1.028	19643

J/g) and *T. ambiguum* (4408 J/g) were intermediate but relatively close to each other (Tab. 3).

A substantial increase in caloric values was detected in 2014 for all species. During this period, energy content ranged from 17729 to 20209 J/g. The highest caloric value was observed in *T. pratense* (20209 J/g), followed by *T. spadiceum* (19643 J/g) and *T. canescens* (19395 J/g). *T. repens* exhibited a similar caloric level (19301 J/g), whereas *T. ambiguum* had the lowest value (17729 J/g) (Tab. 3).

Overall, *T. canescens* showed the highest caloric content in 2013, whereas *T. pratense* exhibited the highest energy concentration in 2014. The notable yearly increase across all species suggests a strong influence of interannual environmental variation – particularly climatic conditions – on the caloric properties of *Trifolium* biomass.

The results read in mg/L (ppm) on the ICP-OES device are shown in the tables below. Elemental differences among the common species of the *Trifolium* L. by year and within species, determined by the Oneb - Way Anova test in the SPSS 17.0 package program. At the same time, the differences between the species whose Tukey HSD values were determined are also stated below (Tab. 3).

Whether there is a significant difference between the years was evaluated with One-Way Anova test and the results are given (Tab. 4 and 5). Among the heavy metals, very significant differences ($P \leq 0.01$) were found in Ni, Zn, Cu, Cr and Mn elements, while significant differences were found in Fe and Al elements at $P \leq 0.05$ level. Since Cd, Pb and Co elements were not between $P \leq 0.01$** and $P \leq 0.05$* values, they do not show significance value. Since 2 comparisons were made on a yearly basis, Tukey HSD values could not be determined. Differences between species and Tukey HSD values are given in Table 4 and 5.

The 2013 ICP-OES measurements of the common Trifolium species were evaluated using a One-Way ANOVA to determine whether heavy-metal concentrations differed significantly among species. The analysis revealed highly significant differences ($P \leq 0.01$) for Ni, Zn, Cu, Cr and Mn, indicating strong interspecific variation in the accumulation of these elements. Significant differences at the $P \leq 0.05$ level were also observed for Fe and Al, suggesting moderate variation among species (Tab. 4).

In contrast, Cd, Pb and Co did not show statistically significant differences ($P > 0.05$), demonstrating that these elements remained relatively uniform across species in 2013 (Tab. 4).

Specifically, nickel ($F=7.758$; $P=0.009$), zinc ($F=21.012$; $P<0.001$), copper ($F=29.637$; $P<0.001$), chromium ($F=12.718$; $P=0.001$), and manganese ($F=16.181$; $P<0.001$) showed the strongest interspecific separation. Iron ($F=6.308$; $P=0.018$) and aluminum ($F=6.070$; $P=0.020$) also varied significantly among species. No significant variation was detected for cadmium ($P=1.000$), lead ($P=0.056$), or cobalt ($P=0.212$) (Tab. 4).

Overall, the results demonstrate that *Trifolium* species differed markedly in their uptake of several essential and trace metals, particularly Zn, Cu, Mn, Cr, and Ni, while other elements such as Cd, Pb, and Co remained comparatively stable across species (Tab. 4).

Significant differences between species was evaluated by using the One-Way Anova test and the results are also given in Table 4, 5. Among the elements, very significant differences ($P \leq 0.01$) were detected in Pb, Fe and Al elements, and at $P \leq 0.05$ level in Mn, Cr and Ni elements. Co, Cd, Cu and Zn elements do not show significance values because they are not between $P \leq 0.01$** and $P \leq 0.05$* values. Tukey HSD results of common species belonging to the *Trifolium* and distributed in the research area according to Cd, Pb, Ni, Fe, Zn, Cu, Al, Cr, Co and Mn elements are shown in (Tab. 4, 5 and 6)

The 2014 ICP-OES measurements of the common *Trifolium* species were evaluated using a One-Way ANOVA to determine interspecific differences in heavy metal accumulation. The analysis revealed highly significant differences ($P \leq 0.01$) for Pb, Fe and Al, indicating strong variation among species for these

Table 4. Evaluation of 2013 Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) Data for *Trifolium* with One-Way Anova test.

		Sum of Squares	Degrees of Freedom	Average of Squares	F	Materiality
Cd	Intergroup	0.000	1	0.000	0.000	1.000
	In-group	0.000	28	0.000		
	Total	0.000	29	-		
Pb	Intergroup	0.000	1	0.000	3.988	0.056
	In-group	0.001	28	0.000		
	Total	0.001	29	-		
Ni	Intergroup	0.007	1	0.007	7.758	0.009**
	In-group	0.024	28	0.001		
	Total	0.030	29	-		
Fe	Intergroup	3308.802	1	3308.802	6.308	0.018*
	In-group	14686.573	28	524.520		
	Total	17995.376	29	-		
Zn	Intergroup	1.789	1	1.789	21.012	0.000**
	In-group	2.384	28	0.085		
	Total	4.173	29	-		
Cu	Intergroup	0.151	1	0.151	29.637	0.000**
	In-group	0.143	28	0.005		
	Total	0.294	29	-		
Al	Intergroup	5264.729	1	5264.729	6.070	0.020*
	In-group	24286.870	28	867.388		
	Total	29551.599	29	-		
Cr	Intergroup	0.028	1	0.028	12.718	0.001**
	In-group	0.063	28	0.002		
	Total	0.091	29	-		
Co	Intergroup	0.016	1	0.016	1.631	0.212
	In-group	0.275	28	0.010		
	Total	0.291	29	-		
Mn	Intergroup	11.570	1	11.570	16.181	0.000**
	In-group	20.022	28	0.715		
	Total	31.593	29	-		

Note: significantly different at 1 % and 5 % (P≤0.01**, P≤0.05*).

elements. Significant differences at the P≤0.05 level were also found for Ni, Cr and Mn, showing moderate interspecific differentiation (Tab. 5).

Lead showed the strongest separation among species (F=16.349; P<0.001), followed by iron (F=5.336; P=0.003) and aluminum (F=5.011; P=0.004). Nickel (F=3.657; P=0.018), chromium (F=3.965; P=0.013) and manganese (F=2.898; P=0.042) also differed significantly among species, although at a lower level of significance (Tab. 5).

In contrast, cadmium (P=0.377), zinc (P=0.197), copper (P=0.178) and cobalt (P=0.236) did not differ

Table 5. Evaluation of 2014 Inductively Coupled Plasma-Optical Emission Spectrometry Data for *Trifolium* with One-Way Anova test.

		Sum of Squares	Degrees of Freedom	Average of Squares	F	Materiality
Cd	Intergroup	0.000	4	0.000	1.103	0.377
	In-group	0.000	25	0.000		
	Total	0.000	29	-		
Pb	Intergroup	0.001	4	0.000	16.349	0.000**
	In-group	0.000	25	0.000		
	Total	0.001	29	-		
Ni	Intergroup	0.011	4	0.003	3.657	0.018*
	In-group	0.019	25	0.001		
	Total	0.030	29	-		
Fe	Intergroup	8288.149	4	2072.037	5.336	0.003**
	In-group	9707.227	25	388.289		
	Total	17995.376	29	-		
Zn	Intergroup	0.866	4	0.216	1.636	0.197
	In-group	3.307	25	0.132		
	Total	4.173	29	-		
Cu	Intergroup	0.063	4	0.016	1.715	0.178
	In-group	0.231	25	0.009		
	Total	0.294	29	-		
Al	Intergroup	13150.319	4	3287.580	5.011	0.004**
	In-group	16401.280	25	656.051		
	Total	29551.599	29	-		
Cr	Intergroup	0.035	4	0.009	3.965	0.013*
	In-group	0.056	25	0.002		
	Total	0.091	29	-		
Co	Intergroup	0.056	4	0.014	1.486	0.236
	In-group	0.235	25	0.009		
	Total	0.291	29	-		
Mn	Intergroup	10.009	4	2.502	2.898	0.042*
	In-group	21.584	25	0.863		
	Total	31.593	29	-		

Note: significantly different at 1 % and 5 % (P≤0.01**, P≤0.05*).

significantly among species, indicating that the concentrations of these elements remained relatively uniform across species in 2014 (Tab. 5).

Overall, the 2014 ANOVA results demonstrate that Trifolium species exhibit pronounced interspecific variation for Pb, Fe and Al, and moderate variation for Ni, Cr and Mn, while Cd, Zn, Cu and Co remain comparatively stable across species. These findings suggest that environmental conditions in 2014 influenced metal uptake patterns differently across species, leading to distinct accumulation profiles for several key elements (Tab. 5).

Tukey HSD analysis was conducted to identify pairwise differences among the five *Trifolium* species for each heavy metal. The results revealed clear interspecific separation for several elements, particularly Al, Fe, Pb, Ni, Cr and Mn. *T. pratense* consistently exhibited the highest concentrations for most metals, whereas *T. ambigium* and *T. canescens* generally showed the lowest values (Tab. 6).

For aluminum (Al), *T. pratense* and *T. repens* formed significantly higher groups, with *T. pratense* displaying the highest mean value, followed by *T. repens*. In contrast, *T. ambigium* and *T. canescens* clustered in the lowest group. A similar pattern was observed for iron (Fe), where *T. pratense* had markedly higher concentrations than the remaining species, particularly *T. ambigium* and *T. canescens*, which again formed the lowest group (Tab. 6).

Lead (Pb) and chromium (Cr) also demonstrated clear differences, with *T. pratense* separating from all other species as the species with the highest accumulation. *T. repens* and *T. spadiceum* occupied intermediate positions, while *T. ambigium* and *T. canescens* showed the lowest Pb and Cr contents (Tab. 6).

Nickel (Ni) displayed a similar trend, with *T. pratense* forming the upper group, *T. repens* and *T. spadiceum* showing moderate values, and *T. ambigium* and *T. canescens* comprising the lowest subgroup. Manganese (Mn) showed a distinctive pattern: *T. repens* was the species with the highest Mn content, followed by *T. pratense*, while *T. ambigium*, *T. canescens* and *T. spadiceum* formed lower value clusters (Tab. 6).

In contrast, some elements – Cd, Co, Zn and Cu – exhibited only minor differences among species, with the Tukey test failing to distinguish clear subgroups. These elements showed relatively uniform distribution across species, consistent with their non-significant ANOVA results (Tab. 6).

Overall, Tukey HSD comparisons confirmed that *T. pratense* and *T. repens* are the species contributing most to interspecific variation in heavy metal accumulation, whereas *T. ambigium* and *T. canescens* consistently exhibited the lowest concentrations. These findings further support the ANOVA results, highlighting substantial species-specific differences in metal uptake within the *Trifolium* genus (Tab. 6).

Statistical graphs were prepared to examine the element distributions among species in more detail (Fig. 3). It was determined that the element Al was found in higher amounts (approx. 60.00 ppm) in the *T. pratense* species. It is seen in lesser amounts on average in *T. repens* and *T. spadiceum* species, and least in *T. ambigium* and *T. canescens* (Fig. 3). It was determined that the Cd was found in higher amounts (0.002 ppm) in the *T. canescens* compared to other species. It was detected less frequently in *T. repens* and *T. pratense*, and at least in *T. ambigium* and *T. spadiceum* species. However, there is no big difference in the amount of Cd among *T. repens*, *T. spadiceum*, *T. ambigium* and *T. pratense* (Fig. 3). The determined amount of the Co was found to be higher (0.100 ppm) in *T. pratense* than in the others and less on average in *T. repens* and *T. spadiceum*, and least in *T. ambigium* and *T. canescens* species. However, there is no big difference in the proportional amount of this element between *T. repens*, *T. spadiceum*, *T. ambigium* and *T. canescens*. The Cr element was found in intense amounts (0.100 ppm) in *T. pratense*, less amount in *T. repens* and *T. spadiceum* on average, and least in *T. ambigium* and *T. canescens* species. The Cu element was found in high amount (0.20, ppm) in *T. pratense*, less in *T. repens* and average *T. spadiceum* and at least in *T. ambigium* and *T. canescens* species. However, there is no big difference between *T .repens*, *T. spadiceum*, *T. ambigium* and *T. canescens* species. The Fe element was found in high amounts (about 45

Table 6. Tukey HSD results for the elements Cd, Pb, Ni, Fe, Zn, Cu, Al, Cr, Co, Mn in species belonging to the *Trifolium* genus.

Species	*T. ambigium*	*T. spadiceum*	*T. pratense*	*T. repens*	*T. canescens*	Significance
Elements			α Sub-identity			
Cd	0.00117	0.00117	0.00117	0.00133	0.00133	0.385
Pb	0.01067	0.01117	0.01183	0.01433	0.02383	0.636
Fe	1.36917	270183	7.85733	12.53350	46.47250	0.861
Zn	0.25967	0.26833	0.27183	0.34183	0.70367	0.245
Cu	0.08467	0.08500	0.09467	0.09600	0.20433	0.229
Al	1.53983	2.79217	7.87133	12.8910	57.64050	0.938
Cr	0.01183	0.02083	0.05133	0.06450	0.10817	0.327
Co	0.00167	0.00233	0.00367	0.00433	0.11083	0.318

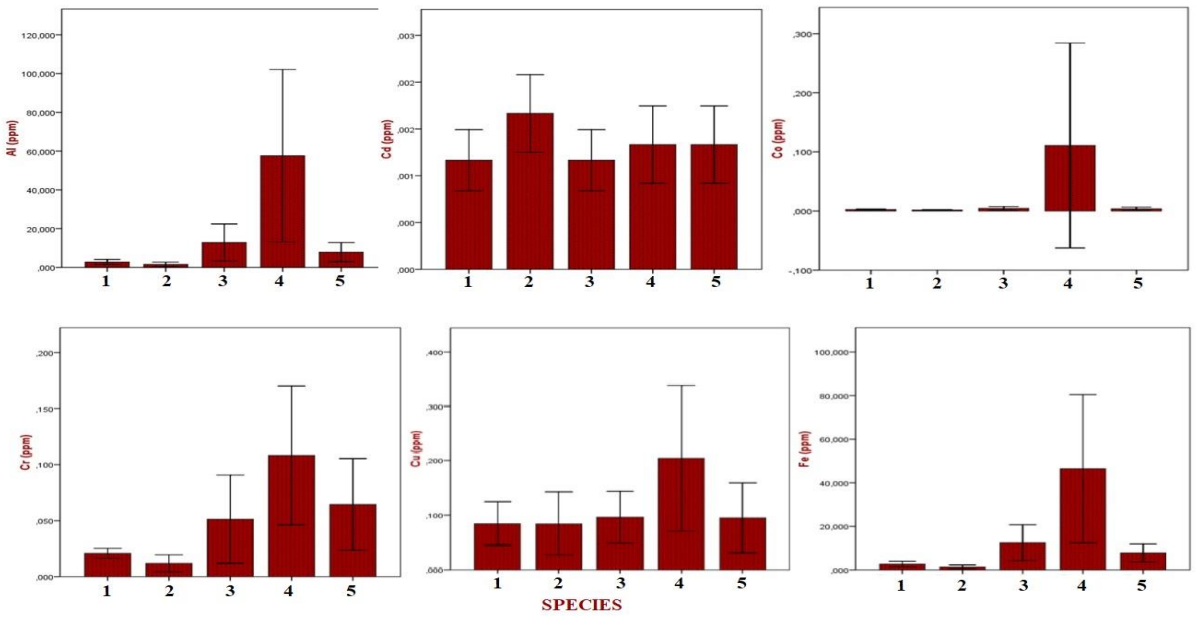


Figure 3. Proportional distribution of Al, Cd, Co, Cr, Cu, Fe elements (ppm) in the chemical content of species belonging to the genus *Trifolium* distributed in the research area: 1. *T. ambigium*, 2. *T. canescens*, 3. *T. spadiceum*, 4. *T. pratense*, 5. *T. repens*.

ppm) in the *T. pratense* in ppm, less in the *T. repens* and average *T. spadiceum*, and the lowest amount in the *T. ambigium* and *T. canescens* species.

It was concluded that the Mn element was abundant in ppm in *Trifolium* repens (approx. 2,000 ppm), on average less in *T. pratense* and *T. spadiceum* species, and at its lowest in *T. ambigium* and *T. canescens* (Fig. 4). The Ni element was abundant in ppm (approx. 0.070 ppm) in the *T. pratense* species, less abundant in the *T. repens* and *T. spadiceum* species on average, and least abundant in the *T. ambigium* and *T. canescens* species. The Pb element was found in high amount (approx. 0.025 ppm) in ppm in *T. pratense* species, less than average in *T. repens* and *T. spadiceum* species, and lowest in *T. ambigium* and *T. canescens*. However, it was noted that there was no big difference in the amount of this element between *T. repens*, *T. spadiceum*, *T. ambigium* and *T. canescens*. The element Zn, in ppm, was abundant in the *T. repens* (0.500-0.100 ppm), on average less in the *T. pratense* and *T. ambigium* species, and at the lowest amount in the *T. spadiceum* and *T. canescens*. However, there was no big difference in the amount of this element between *T. pratense*, *T. spadiceum*, *T. ambigium* and *T. canescens*.

Considering the hygroscopic humidity rates of the five *Trifolium* species prevalent in the Çamlıhemşin district of Rize Province and included in this study, the 2013 data revealed that *T. pratense*, *T. canescens*, and *T. ambiguum* were the most productive species. It has been found that the productivity of these species is in the range of 125-105 gr/m^2. The productivity of *T. repens* and *T. spadiceum* is lower in the range of 85-32 gr/m^2.

The amount of hygroscopic moisture in these species; It was determined that it was higher in *T. canescens*, *T. ambiguum* and *T. pratense*, than in *T. repens* and *T. spadiceum*. Based on this, according to the 2013 evaluation, the order of *T. canescens* > *T. ambigium* > *T. pratense* > *T. repens* > *T. spadiceum* was determined in terms of productivity. Comparing the 2014 data with the 2013 data, it was observed that there was not much difference in terms of productivity between the species, while *T. pratense*, *T. canescens* and *T. ambiguum* were the species with the highest productivity. The productivity of *T. repens* and *T. spadiceum* species was lower in comparison with first three.

The amount of hygroscopic moisture in the species *T. canescens*, *T. ambiguum* and *T. pratense* was higher than in *T. repens* and *T. spadiceum*. Based on this, according to the 2013 evaluation in terms of productivity, the order *T. canescens* > *T. ambigium* > *T. pratense* > *T. repens* > *T. spadiceum* emerged. We believe that this difference is related to the habitat where the plants live, as well as the ecological characteristics of the plants and the aspect and direction of the slope.

It seems that the hygroscopic moisture rate in these

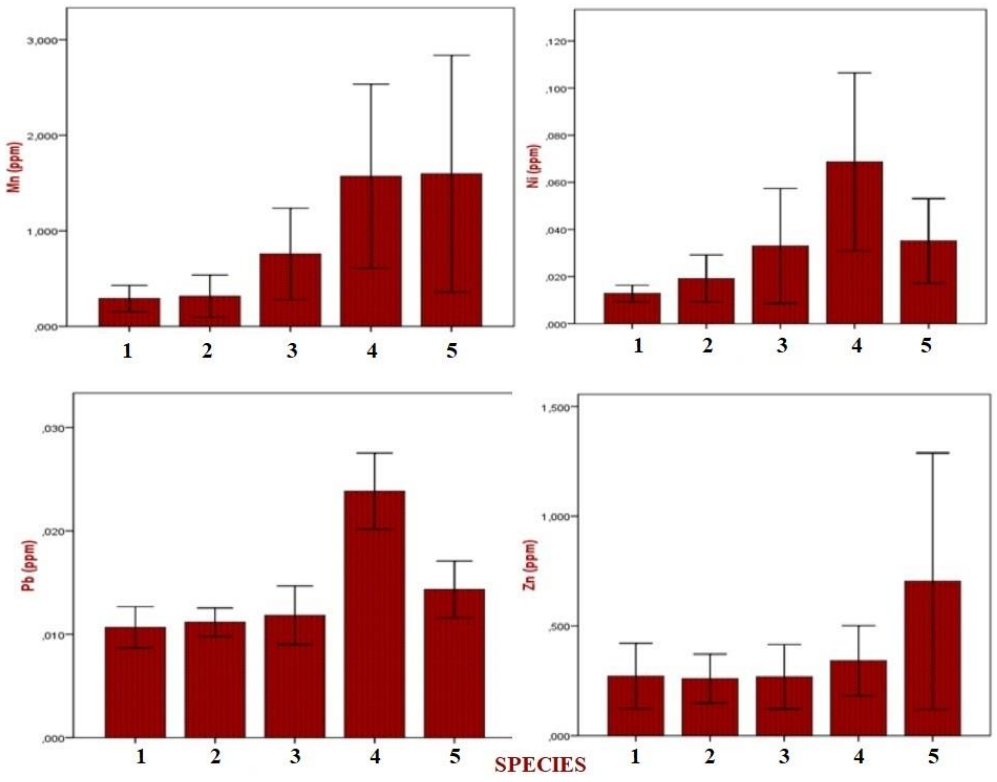


Figure 4. Proportional distribution of Mn, Ni, Pb, Zn elements (ppm) in the chemical content of species belonging to the genus *Trifolium* distributed in the research area: 1. *T. ambigium*, 2. *T. canescens*, 3. *T. spadiceum*, 4. *T. pratense*, 5. *T. repens*.

plants changes in parallel with the productivity rate.

A.I. Mayilov and V.V. Atamov (1985) examined the energy content of aboveground phytomass in Stipetum communities in Gobustan (Azerbaijan). Their findings showed that 1 gram of fodder material contains on average 5.7% protein (256.5 cal), 1.4% fat (116.5 cal), 36.1% cellulose (1044.0 cal), and 46% nitrogen-free extractive substances (1702 cal). Overall, the total energy value of 1 gram of this mass is 3118.7 cal. The researchers estimated that, during the flowering period, the phytomass produced in a 1-hectare area by these communities accumulates approximately 7.5 million calories of energy.

As a result of the analysis of Calorie content in 2013 samples the amount of energy obtained from 1 gram of *T. canescens* is 4714 joules/gr, the amount of energy obtained from 1 gram of *T. repens* is 4547 joules/gr, the amount of energy obtained from 1 gram of *T. pratense* is 4412 joules/gr, the amount of energy obtained from 1 gram of *T. ambigium* is 4408 joules/gr, the amount of energy obtained from 1 gram of *T. spadiceum* is 4369 joules/gr. Based on these analysis, it appears that the *T. canescens* species has the highest amount of calories, in comparison with *T. spadiceum*.

As a result of the analysis of 2014 samples, the amount of energy obtained from 1 gram of *T. pratense* is 20209 joules/gr, *T. spadiceum* – 19643 joules/gr, *T. canescens* – 19395 joules/gr, *T. repens* – 19301 joules/gr, *T. ambigium* – 17729 joules/gr. As a result of the current study, *T. pratense* was found to be more productive than *T. canescens* compared to 2013. *T. spadiceum*, which had low productivity in 2013, showed higher productivity in the second year in the ranking *T. pratense* > *T. spadiceum* > *T. canescens* > *T. repens* > *T. ambigium*.

CONCLUSION

In the study of some important meadow clover (*T. pratense* L.) varieties in the Erzurum region, F. Tosun and M. Altın [1986] tested the varieties on loamy soils, under irrigation conditions, with four replications using a random block trial design. In all three trials, the lowest yield was obtained in the establishment year and the highest yield was obtained in the second year of the trial.

As the years progressed, depending on the varieties, there was a more or less general decrease, and even in the 3rd and 4th years, some varieties were completely withdrawn from testing. In all three experiments, the average yields of the varieties decreased in parallel with the following years. For example, 1504.0 kg per decare in the second year of the first meadow clover trial. The average of the varieties increased to 918.7, 807.2, 678.3 kg in 1969, 1970, 1971, respectively, and in the second attempt, 1474.6, 830.8, 769.9 and 720.6 kg, respectively and the average weight was determined as 900 kg.

It is also seen that studies on productivity have been carried out not only on herbaceous plants but also on woody plants. Kuzugüdenli E. and Özkan K. [2010] examined the relationships between the productivity of red pine (*Pinus brutia* Ten.) and habitat characteristics in the Sütçüler region of the Mediterranean region in 2010.

M. Başbag et al., [2011] studied the quality characteristics of several clover species (*Trifolium* spp.) collected from their native ranges in the Southeastern Anatolia region. They found that *T. repens*, *T. resupinatum*, and *T. repens* rank highest in quality, followed by *T. nigrescens*, *T. campestre*, *T. fragiferum*, and *T. tomentosum*, respectively. The annual change in species in the same area can be explained by seasonal climate changes. For energy production and maximum biomass yield, a longer interval between harvests is recommended, for example, at least 90 days [Cevheri et al., 2013; Johannees et.al., 2025].

According to results, we observed that the highest amount of elements was in the *T. pratense* species, followed by *T. repens*, *T. spadiceum*, *T. ambigium*, *T. canescens* species. *Trifolium* L. generally has high quality in terms of productivity. The *T. pratense* species belonging to the genus has abundant leaves, its herbage yield and nutritional value are quite high. It is a species with a decreasing population frequency.

The unconscious collection of many plants with economic and medicinal value from nature causes plant species to disappear from nature. It is necessary to inform the local people about avoiding overgrazing and grazing the pastures in a balanced manner in accordance with the load.

To ensure the sustainable use of *Trifolium* species in high-mountain ecosystems, grazing pressure should be managed through rotational systems and local awareness programs. Regular monitoring of soil and plant heavy metal concentrations is essential, particularly for *T. pratense* and *T. repens*, which tend to accumulate higher metal levels. Species such as *T. canescens* and *T. ambiguum*, showing both high productivity and lower contamination, should be prioritized for pasture improvement and conservation. Future studies should expand on genetic, physiological, and environmental factors influencing metal uptake and forage quality across varying altitudes and soil types.

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REFERENCE

Acar Z., Ayan I. Forage Crops Culture. Ondokuz Mayıs University Faculty of Agriculture Publications, 2012, No. 2, Samsun, Türkiye.

Angon P.B. et al. (2024) Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. *Heliyon*, 10(7): e28357.

Atamov V. Rize'nin Bitki Çeşitliliği. Duvar yayınları, 2021, ISBN: 978-625-7502-20-7.

Atamov V.V., Mailov A.I., Gafarova Z.J., Mamedov S.Y. (1985) A little biological circulation of organic mass, nitrogen, main ash elements and quantity energy in overhead phytomass in the *Stipetum* phytocenoses of Gobustan. *Izvestiya AN Azerb. SSR, Seriya Biol. Nauk*, 1: 9-16.

Başbağ M., Çaçan E., Aydın A., Sayar M.S. (2011) Güneydoğu Anadolu Bölgesi doğal alanlarında toplanan bazı fiğ türlerinin ot kalite özelliklerinin belirlenmesi. *I. Ali Numan Kıraç Tarım Kong. Fuarı*, 27–30 Nisan.

Cevheri C., Küçük Ç., Avcı M., Atamov V. (2013) Element content, botanical composition and nutritional characteristics of natural forage of Şanlıurfa. *J. Food Agric. Environ.*, 3-4: 790-794.

Davis P.H. Flora of Turkiye and the East Aegean Islands. Edinburgh University Press, 1970, Vol. 3, 628 s., 1-628. ISBN: 0 85224 154 2.

Fisher R.A. (1934) Statistical methods for research workers.

Greveniotis V., Bouloumpasi E., Skendi A., Korkovelos A., Kantas D., Ipsilandis C.G. (2025) Evaluation and stability of red and white *Trifolium* species for nutritional quality in a Mediterranean Environment. *Agriculture*, 15(4): 391. https://doi.org/10.3390/agriculture15040391.

Hasan M.K., Jahan I., Suravi T.I., Al Hasib Imon M.A., Shopan J., Ahammed G.J. (2025) Heavy metals

and microplastics derived from laboratory effluents enhance toxicological risks to the ecosystems of canals in Bangladesh. *Environ. Sci. Pollut. Res. Int*., 32(2): 949-962. doi: 10.1007/s11356-024-35758-6.

Hsu J.C. (1997) Multiple Comparisons Theory and Methods. *Comput. Stat. Data Anal.*, 25(2): 241-243.

Johannes L.P., Minh T.T.N., Son N.V., Tung D.T., Viet T.D., Xuan T.D. (2025) Agronomic and Utilization Potential of Three Elephant Grass Cultivars for Energy, Forage, and Soil Improvement in Vietnam. *Crops*, 5(5): 70. https://doi.org/10.3390/crops5050070

Karahan F. (2023) Evaluation of trace element and heavy metal levels of some ethnobotanically ımportant medicinal plants used as remedies in Southern Turkiye in terms of human health risk. *Biol. Trace. Elem. Res.*, 201: 493-513. https://doi.org/10.1007/s12011-022-03299-z.

Kahvecioğlu Ö., Kartal G., Güven A., Timur S. (2006) Environmental effects of metals-I. ITU Metallurgical and Materials Engineering Chamber Metallurgy Journal, 136: 4-10 [Kahvecioğlu Ö., Kartal G., Güven A., Timur S. (2006) Metallerin Çevresel Etkileri-I. İTÜ Metalurji ve Malzeme Mühendisliği Odası Metalurji Dergisi, 136: 4-10]

Kuzugüdenli E., Özkan K. (2010) Relationships between productivity and habitat characteristics of red pine (*Pinus brutia* Ten.) in Sütçüler district of the Mediterranean region. *Süleyman Demirel Univ. For. Fac. J.*, 16-29 [Kuzugüdenli E., Özkan K. (2010) Akdeniz Bölgesi Sütçüler Yöresinde kızılçamın (*Pinus brutia* Ten.) verimliliği ile yetişme ortamı özellikleri arasındaki ilişkiler. *Süleyman Demirel Üniv. Orm. Fak. Der.*, 16-29]

Mayilov A.I., Atamov V.V. (1985) The value of fodder grass and the capacity of types of pastures of Azerbaijan. *Dokl. Akad. Nauk Azerb. SSR*, 1984, C.XL N 2, s. 65-68. [Маилов А.И., Атамов В.В. (1985) Ценность кормовых трав и продуктивность типов пастбищ Азербайджана. *Докл. Akad. Наук Азерб. CCP*, 1984, C.XL, N 2, c. 65-68.] (in Russian)

Özkan, K., & Kuzugüdenli, E. (2010) The relations between site index of brutian pine (*Pinus brutia* Ten.) and ecological site factors in Sütçüler district from the Mediterranean region. *Turk. J. For.*, 11(1): 16-29.

Sharma R.K., Agrawal M. (2005) Biological effects of heavy metals: An overview. *EMAS*, 120(1-3): 71-79.

Snedecor G.W., Cochran W.G. Statistical methods, 8thEdn. Ames: Iowa State Univ. Press Iowa, 1989, 54, 71-82.

Tosun F., Altın M., (1986) Pastureland Culture and Utilization Methods. Ondokuz Mayıs Unive. Publ. No: 5 (2nd Edition). Samsun. [Tosun, F., Altın, M. (1986) Çayır-Mer'a Kültürü ve Bunlardan Faydalanma Yöntemleri. Ondokuz Mayıs Üniver. Yay. No: 5 (2. Baskı). Samsun]

Tukey J. W. (1949) Comparing individual means in the analysis of variance. *Biometrics*, 99-114.

U.S. Environmental Protection Agency (1996) Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices. EPA SW-846. Retrieved from https://www.epa.gov/sites/default/files/2015-12/documents/3052.pdf

Çamlıhemşin (Rize, Türkiyə) yüksək dağlıq hissələrində bəzi *Trifolium* L. (Fabaceae) növlərinin yem dəyərinin və ağır metal tərkibinin tədqiqi

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Bu tədqiqatda Çamlıhemşin (Rize, Türkiyə) yüksək dağ otlaqlarında yayılmış *Trifolium* (Fabaceae) cinsinin seçilmiş növlərinin yem məhsuldarlığı, yem dəyəri (kalorisi) və ağır metal tərkibi araşdırılmışdır. Verçenik, Gito, Kavron, Çeymakçur, Elevit, İncesu və Çat yaylalarında 2013-2014-cü illərdə çiçəkləmə dövründə çöl işləri aparılmışdır. Tez-tez rast gəlinən beş takson (*Trifolium pratense*, *T. repens*, *T. ambiguum*, *T. spadiceum* və *T. canescens*) toplanmış, herbarizasiya edilmiş və təhlil edilmişdir. Məhsuldarlıq və hiqroskopik rütubət biokütlə nümunəsi, kalorili dəyərlər isə bomb kalorimetri ilə ölçülmüşdür. Ağır metal konsentrasiyaları (Al, Cd, Pb, Ni, Cr, Cu, Fe, Zn, Mn, Co) həzm (hazırlama) prosesindən sonra ICP-OES istifadə edərək ölçüldü. Nəticələrə görə *T. canescens* və *T. ambiguum* ən yüksək məhsuldarlıq və rütubət, *T. spadiceum* isə ən aşağı məhsuldarlıq nümayiş etdirmişdir. Kalori dəyərləri 2013-cü ildə 4369–4714 J/q və 2014-cü ildə 17729–20209 J/q arasında dəyişmişdir, ən yüksək dəyərlər *T. pratense* və *T. canescens* növlərində qeydə alınmışdır. Ağır metal konsentrasiyalarında əhəmiyyətli növlərarası və illərarası fərqlər müşahidə edilmişdir, *T. pratense* daha yüksək səviyyədə Al, Fe, Cu, Cr, Ni, Pb və Co metalları, *T. repens* isə daha yüksək Mn və Zn toplamışdır. Bu nəticələr təsdiq edir ki, *Trifolium*

növlərinin regionda qiymətli yem ehtiyatları vardır. Ağır metalların toplanmasında müxtəliflik yüksək dağlıq otlaqlarda ekoloji və antropogen təsirlərin monitorinqinin vacibliyini vurğulayır.

Açar sözlər: *biokütlə nümunəsi, kalori dəyəri, yem resursları, ICP-OES, variabillik*

Исследование кормовой ценности и содержания тяжелых металлов в некоторых таксонах рода *Trifolium* L. (Fabaceae) в высокогорных районах Чамлыхемшина (Ризе, Турция)

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В данном исследовании изучается кормовая продуктивность, кормовая ценность (калорийность) и содержание тяжелых металлов в отдельных таксонах рода *Trifolium* (Fabaceae), произрастающих на высокогорных пастбищах Чамлыхемшина (Ризе, Турция). Полевые исследования проводились в период цветения 2013–2014 годов на плато Верченик, Гито, Каврон, Чаймакчур, Элевит, Инджесу и Чат. Были, гербаризированы и проанализированы пять часто встречающихся виды (*Trifolium pratense*, *T. repens*, *T. ambiguum*, *T. spadiceum* и *T. canescens*). Продуктивность и гигроскопическая влажность определялись путем отбора проб биомассы, а калорийность измерялась с помощью бомбового калориметра. Концентрации тяжелых металлов (Al, Cd, Pb, Ni, Cr, Cu, Fe, Zn, Mn, Co) количественно определялись с помощью ICP-OES после влажного сбраживания (подготовительный процесс). Результаты показали, что *T. canescens* и *T. ambiguum* продемонстрировали самую высокую продуктивность и влажность, тогда как *T. spadiceum* – самую низкую. Калорийность варьировала от 4369 до 4714 Дж/г в 2013 г. и от 17729 до 20209 Дж/г в 2014 г., причем самые высокие значения были у *T. pratense* и *T. canescens*. Наблюдались значительные межвидовые и межгодовые различия в концентрациях тяжелых металлов: *T. pratense*, как правило, накапливал более высокие уровни Al, Fe, Cu, Cr, Ni, Pb и Co, в то время как *T. repens* демонстрировал более высокое содержание Mn и Zn. Полученные результаты подтверждают, что виды рода *Trifolium* являются ценными кормовыми ресурсами в регионе. Изменчивость накопления тяжёлых металлов подчёркивает важность мониторинга экологического и антропогенного воздействия на высокогорные пастбища.

Ключевые слова: *отбор проб биомассы, калорийность, кормовые ресурсы, ICP-OES, изменчивость*