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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Macro, Micro Element and Heavy Metal Contents of *Astragalus* Taxa Collected from Nature

Doğadan Toplanan *Astragalus* Taksonlarının Makro, Mikro Element ve Ağır Metal İçeriklerinin Belirlenmesi

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Abstract

The aim of the research is to determine the macro (Ca, Mg, P and K), micro (Fe, Mn, Cu, Zn and Be) element and heavy metal (Al, Cr, Ni, Se and Cd) contents of some *Astragalus* taxa collected from nature. Nine *Astragalus* taxa (*Astragalus gummifer, A. compactus, A. lineatus* var. *longidens, A. aureus, A. onobrychis, A. declinatus, A. lineatus* var. *lineatus, A. oocephalus* subsp. *stachyophorus, A. inereus*) collected from the natural areas of Bingöl province were used as the plant material of the research. *Astragalus* taxa were collected from the central villages of Bingöl province in June 2018, during the flowering period of the plants. Macro, micro element and heavy metal contents of the collected and dried plant samples were determined with the help of NIRS (Near Infrared Spectroscopy) and ICP-MS (Inductively Coupled Plasma - Mass Spectometry) devices. As a result of the analysis, the average Ca content of *Astragalus* taxa was determined as % 1.63, Mg content % 0.36, P content % 0.27, K content % 1.49, Fe content 2436 mg kg^{-1}, Mn content 153.7 mg kg^{-1}, Cu content 84.5 mg kg^{-1}, Zn content 14.08 mg kg^{-1}, Be content 0.10 mg kg^{-1}, Al content 2535 mg kg^{-1}, Cr content 7.67 mg kg^{-1}, Ni content 9.99 mg kg^{-1} and Se content 2.19 mg kg^{-1}. In general, the highest Ca, Mg, Fe, Mn, Al, Cr and Ni contents were determined in A. *declinatus*, the highest P and K contents were determined in *A. oocephalus* subsp. *stachyophorus*, the highest Cu content was determined in *A. cinearus*, the highest Zn content was determined in *A. lineatus* var. *longidens*, the highest content Be was determined in *A. gummifer* and the highest Se content was determined in *A. compactus*. Cd content could not be detected in any *Astragalus* taxa. As a result of the study, it was concluded that *Astragalus* taxa, which are abundant in the natural flora of our country, can be used as a source of roughage in animal nutrition and can meet the macro and micro element needs of animals.

Keywords: *Astragalus* sp, Macro elements, Micro elements, Minerals, Heavy metals.

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Öz

Bu çalışma doğal alanlardan toplanan bazı *Astragalus* taksonlarının makro (Ca, Mg, P ve K), mikro (Fe, Mn, Cu, Zn ve Be) ve ağır metal (Al, Cr, Ni, Se ve Cd) içeriklerinin belirlenmesi amacıyla yürütülmüştür. Bingöl ili doğal alanlarından toplanan dokuz adet *Astragalus* taksonu (*Astragalus gummifer, A. compactus, A. lineatus* var. *longidens, A. aureus, A. onobrychis, A. declinatus, A. lineatus* var. *lineatus, A. oocephalus* subsp. *stachyophorus, A. inereus*) araştırmanın bitkisel materyali olarak kullanılmıştır. *Astragalus* taksonları 2018 yılının haziran ayında, bitkilerin çiçeklenme döneminde Bingöl ilinin merkez köylerinden toplanmıştır. Toplanıp kurutulan bitki örneklerinin makro, mikro element ve ağır metal içerikleri NIRS (Near Infrared Spectroscopy) ve ICP-MS (Inductively Coupled Plasma - Mass Spectometry) cihazları yardımıyla belirlenmiştir. Analizler sonucunda *Astragalus* taksonlarının ortalama Ca içeriği % 1.63, Mg içeriği % 0.36, P içeriği % 0.27, K içeriği % 1.49, Fe içeriği 2436 mg kg^{-1}, Mn içeriği 153.7 mg kg^{-1}, Cu içeriği 84.5 mg kg^{-1}, Zn içeriği 14.08 mg kg^{-1}, Be içeriği 0.10 mg kg^{-1}, Al içeriği 2535 mg kg^{-1}, Cr içeriği 7.67 mg kg^{-1}, N içeriği 9.99 mg kg^{-1} ve Se içeriği 2.19 mg kg^{-1} olarak belirlenmiştir. Genel olarak en yüksek Ca, Mg, Fe, Mn, Al, Cr ve Ni içerikleri *A. declinatus*, en yüksek P ve K içerikleri *A. oocephalus* subsp. *stachyophorus*, en yüksek Cu içeriği *A. cinearus*, en yüksek Zn içeriği *A. lineatus* var. *longidens,* en yüksek Be içeriği *A. gummifer* ve en yüksek Se içeriği de *A. compactus* taksonunda tespit edilmiştir. *Astragalus* taksonlarında Cd içeriği tespit edilmemiştir. Çalışma sonucunda, Ülkemiz doğal florasında bol miktarda bulunan *Astragalus* taksonlarının hayvan beslemede kaba yem kaynağı olarak kullanılabileceği ve hayvanların makro ve mikro element ihtiyacını karşılayabileceği sonucuna varılmıştır.

Anahtar kelimeler: *Astragalus* sp., Makro elementler, Mikro elementler, Mineraller, Ağır metaller.

1. Introduction

Plants are very important because of their nutritional worth, and they have always been a primary source of medicines throughout human history. Herbal supplements, botanicals, nutraceuticals, and medication formulations account for 30 to 40% of today's conventional pharmaceuticals used in the medicinal and curative characteristics of various plants (Schulz et al., 2001). Essential metals and minerals are important and play a unique role in the structuring of living systems' functional and structural integrity (Wadhwa, 2015).

The genus *Astragalus* L. is a member of the *Fabaceae* (*Leguminosae*) family, *Leguminosae* which is one of the largest plant families on earth and second big family after Asteraceae. Many *Astragalus* L. taxa have high medicinal and economic values. This genus is the biggest genus in the world in terms of number of species, this genus is represented more than 2700 annual or perennial taxa in the World (Podlech and Zarre, 2013); in Turkey, *Astragalus* is represented more than 470 taxa with about 50% endemism ratio (Aytac et al., 2012; Ekici et al., 2015; Donmez and Aydin, 2018).

The taxa of *Astragalus* have a broad spectrum of usages. Many *Astragalus* taxa are helpful as forage plants, to management erosion, as medicinal plants or as ornamentals; besides some *Astragalus* taxa have a wide range of applications in pharmaceuticals, as thickening agents in foods, and may have applications in controlling cancer cells. Some of *Astragalus* taxa used as feed for animals, as fuel and they are indicator plants for selenium.

The literature also shown that the majority of edible gum derived from the *Astragalus* genus has a wide range of uses in the pharmaceutical, cosmetic, and food industries (Verbeken et al., 2003; Jani et. al., 2009; Azarikia and Abbasi, 2010; Gorji et al., 2011; Nejatian et al., 2013). Some *Astragalus* taxa are used as animal feeds, medications and pharmaceuticals, erosion control, bee pastures, dye and textile industries (Gruenwald et al., 1998). *Astragalus* taxa are often grazed by ruminants in steppes with low precipitation levels and Alpines with cold temperatures. The literature shows that, some *Astragalus* taxa has antioxidant, hepatoprotective, anti-inflammatory, antitumor, anti-fatigue, anticancer, antiviral, antibacterial, immune system enhancing, immune stimulant, antiinfective some viruses, adaptogen, cardiotonic, diuretic, hypotensive, immunomodulator, hypoglycaemic, circulatory stimulant, vasodilator and expectorant effects (Ma et al., 2011; Zhao et al., 2011); also uses to treatment chronic phlegmatic disorders, general gastrointestinal disturbances including stomach ulcer, chronic diarrhea and intestinal inflammation (Yang et al., 2014). Radix Astragali drog provide important protection against heart, intestine, kidney, liver, brain, kidney, and lung injury in various models of oxidative stress-related disease (Shahzad et al., 2016). In many ethnobotanical practices around the world, these vital plants are widely used as medicine, food, fodder, fuel, and ornamental plants (Amiri et al., 2020).

Astragalus taxa are used in many different ways. Most of *Astragalus* taxa have high nutritional values. It is known to be grazed by animals during their maturity period. Some *Astragalus* taxa are uprooted with their roots (Manga et al., 2003). Some *Astragalus* contribute to the protection and formation of the soil, especially by growing in steppe and areas exposed to erosion. *Astragalus* species that grow in natural habitats not only provide feed sources for domestic and wild animals, but also create shelter areas for wild animals (Basbag et al., 2017). Especially in the form of bush and semi-shrub, *Astragalus* species protect some weeds around them due to their thorny structures during grazing in pastures in spring and summer. As a result of the softening of the thorns and the moistening of the dried grass with the rain in autumn, the need for roughage is supplied from these dried grasses together with the reduced grass in the pasture.

The number of nutrients studies conducted on this big genus, is still too few. Therefore, the main goal of current study was to determine macro, micro element and heavy metal contents and nutritive value of studied *Astragalus* taxa in order to support sufficient baseline data for subsequent works.

2. Material and Method

Plant materials are collected at the localities and dates indicated in *Table 1*, by O. Kilic, E. Cacan and K. Kokten. Plants were identified according to Volume-3 of Flora of Turkey and East Aegean Islands (Davis, 1982).

Plant samples belonging to *Astragalus* taxa were taken at the locations and dates indicated in *Table 1*, during the flowering period and according to the random sampling method. An average of 200 g of green grass was taken from each location. The plant samples taken were dried at 70°C for 48 hours. After the dried samples were ground with the help of a hand mill, they were sieved through a 1 mm sieve and made ready for analysis. Ca, Mg, P and

K contents of Astragalus taxa were determined with the help of NIRS (Near Infrared Spectroscopy, Foss Model 6500) device (Engin and Mut, 2018; Basbag et al., 2019) at Ondokuz Mayıs University Faculty of Agriculture. Fe, Mn, Cu, Zn, Be, Al, Cr, Ni, Se and Cd contents were determined with the help of ICP-MS device in Bingol University Central Laboratory Application and Research Center.

Table 1. The localities and dates where the Astragalus taxa were collected

Plant samples	Localities	Collected Date
Astragalus gummifer	Bingöl Center Yolcati vicinity	June 2018
Astragalus compactus	Bingöl Center Yolcati vicinity	June 2018
Astragalus lineatus var. *longidens*	Bingöl Center Kuruca vicinity	June 2018
Astragalus aureus	Bingöl Center Kuruca vicinity	June 2018
Astragalus onobrychis	Bingöl Center Kuruca vicinity	June 2018
Astragalus declinatus	Bingöl Center Kuruca vicinity	June 2018
Astragalus lineatus var. *lineatus*	Bingöl Center Hesarek vicinity	June 2018
Astragalus oocephalus subsp. *stachyophorus*	Bingöl Center Asagikoy vicinity	June 2018
Astragalus cinereus	Bingöl Center Asagikoy vicinity	June 2018

3. Result and Discussion

Plants, which have a strong connection with the soil rich in plant nutrients, continue their lives more efficiently and successfully. Each plant has its own optimum nutrition range as well as a minimal nutrient demand level. Plants begin to show signs of nutrient shortage below this threshold. Due to toxicity, excessive nutrient intake might potentially result in poor growth. As a result, the amount of nutrients applied and their positioning are critical.

Ca (Calcium) has an important role in the development and plasticity of the cell wall membrane, which influences normal cell division by maintaining cell integrity and membrane permeability. Without enough calcium, which is needed to produce solid cell walls in the form of calcium pectate, freshly emerging leaves may stick together at the margins, causing ripping as the leaves spread and unfurl. The stem structure may also be weakened as a result of this. Mineral substances have an important place in the nutrition of animals. Calcium is the third most used plant nutrient in plants. It is a part of the plant cell wall and therefore it is known as the plant nutrient element that regulates the cell wall structure.

The macro element contents of the studied *Astragalus* taxa are shown in *Table 2*. Calcium ratios of taxa varied between 0.69-2.02%; and average Ca ratio is 1.63%. *Astragalus declinatus* has highest Ca ratio (2.02%). The lowest calcium rate is seen in *Astragalus compactus* (0.69%) (*Table 2*). Calcium is high in *Astragalus meridioalis* seeds (Rahimi et al., 2017). Calcium play significant role in maintaining strong bones and teeth. It is also required for appropriate heart muscle activity, blood coagulation, milk clotting, and cell permeability modulation (Smith, 1987). Calcium deficiency back pain, causes rickets, indigestion, osteoporosis, irritability and cramping of the uterus (Diaz-Gomez et al., 2003). In a study calcium ratios of *Astragalus hamosus* from different localities varies between 1.25-1.99% (Basbag et al., 2019). In another research Ca ratios in different *Astragalus* taxa was ranged from 0.93-1.18% (*Astragalus pinetorum* subsp. *declinatus* (1.18%), *Astragalus densifolius* (1.09%), *Astragalus lagurus* (0.94%), *Astragalus aduncus* (0.93%) (Parlak, 2019).

Mg (Magnesium) is an important element and part of the chlorophyll molecule, the deficiency symptom of interveinal chlorosis first appears in older leaves. Magnesium has many roles including supporting the functioning of the immune system; synthesis of proteins and in muscle contraction and cell integrity. The Mg ratios of the studied taxa are very close to each other; *Astragalus declinatus* has highest Mg ratio (0.47%) and the lowest calcium rate is seen in *Astragalus cinereus* (0.24%) (*Table 2*). In a study, Mg ratios of *Astragalus hamosus* from different localities varies between 0.31-0.46% (Basbag et al., 2019). In another study, Mg ratio was determined highest (1.51%) in *Astragalus microcephalus* (Parlak, 2019).

Table 2. Macro element contents of Astragalus taxa (%)

Plant samples	Ca	Mg	P	K
Astragalus gummifer	1.53	0.41	0.25	1.21
Astragalus compactus	0.69	0.28	0.27	1.55
Astragalus lineatus var. *longidens*	1.45	0.33	0.32	2.49
Astragalus aureus	1.81	0.39	0.30	1.62
Astragalus onobrychis	1.99	0.41	0.19	0.68
Astragalus declinatus	2.02	0.47	0.17	0.58
Astragalus lineatus var. *lineatus*	1.80	0.38	0.29	1.27
Astragalus oocephalus subsp. *stachyophorus*	1.64	0.36	0.36	2.51
Astragalus cinereus	1.71	0.24	0.25	1.55
Average	**1.63**	**0.36**	**0.27**	**1.49**

Because of K (Potassium) is an enzyme activator that increases metabolism, its presence is critical for plant growth, K has been demonstrated to help plants withstand disease, increase grain and seed size, and improve the quality of fruits and vegetables. K ratios of taxa varied between 0.58-2.51%; and average K ratio is 1.49%. *Astragalus oocephalus* subsp. *stachyophorus* has highest K ratio (2.51%); the lowest K rate is in *Astragalus declinatus* (0.58%) (*Table 2*). K shortage causes nervous irritability, mental confusion, low blood sugar, sleeplessness, and coma because it aids in the release of molecules that act as nerve impulses and control heart rhythms (Gaeta and Hider, 2005).

Phosphorus (P) is a mineral that is found in plants' nucleus and is involved in protein synthesis control. Phosphorus is essential for the formation of new tissue and cell division. Plants carry out complicated energy transmissions, which necessitates the use of phosphorus. P is very effective especially in the early development stages of the plant and plays an important role in flowering, root development, seed and fruit formation. It affects the energy balance with the circulation and exchange of substances in the plant. P ratios of the studied taxa are also very close to each other; and detected an average 0.27% (*Table 2*). In a study P ratio of *Astragalus hamosus* collected from different localities varies between 0.20-0.45% (Basbag et al., 2019). In another study the highest phosphorus content was 2.82% from *Astragalus aduncus*, this followed by *Astragalus densifolius* with a rate of 0.92%, and *Astragalus lagurus* with the lowest rate of 0.84% (Parlak, 2019).

Motsara and Roy (2008) reported that the limit values Ca ratio should be between 0.1-1.0%, Mg ratio between 0.1-0.4%, P ratio between 0.2-0.5% and K ratio between 1.0-5.0% in plants. It was observed that Ca contents (%1.63) of Astragalus taxa were high, while other macro element contents (Mg %0.36, P %0.27, K %1.49) were within or close to the limit values reported by Motsara and Roy (2008).

The micro element contents of the studied *Astragalus* taxa are shown in *Table 3*. Iron (Fe) is essential in the heme enzyme system in plant metabolism. Iron is one of the most deficient micro-elements in the world. Fe is less mobile in the plant and is required for photosynthesis processes in conjunction with plant respiration. It plays an important role in enzyme activities and chlorophyll synthesis. Iron also serves as an activator for biochemical processes such as photosynthesis, respiration, and symbiotic nitrogen fixation (Hawkes et al., 1985). On the basis of the base normal levels of iron in plants (50-300 mg kg^{-1}), the analyzed plant accumulates enough iron at all stages of growth (Fox and Guerinot, 1998). The Fe content in *Astragalus onobrychis* subsp. *chlorocarpus* increased during vegetation and showed maximum in the seed forming stage of growth, 343.02 mg kg^{-1} (Miladinovic et al., 2011). The Fe content of *Astragalus mollissimus* changes significantly throughout the growing season, peaking in February at 400 mg kg^{-1} (Leand, 1986). In their investigation, Sheded et al. found Fe levels ranging from 261 to 1239 mg kg^{-1} in a variety of Egyptian medicinal herbs (Sheded et al., 2006). In this research, highest Fe ratio (7338 mg kg^{-1}) in *Astragalus declinatus* and the lowest Fe rate is seen in *Astragalus oocephalus* subsp. *stachyophorus* (200 mg kg^{-1}) (*Table 3*).

Table 3. Micro element contents of Astragalus taxa (mg kg^{-1})

Plant samples	Fe	Mn	Cu	Zn	Be
Astragalus gummifer	794	48.5	71.1	1.59	0.42
Astragalus compactus	2264	108.4	62.3	0.00	0.14
Astragalus lineatus var. *longidens*	2478	132.6	88.8	34.22	0.00
Astragalus aureus	3096	229.1	87.6	13.36	0.06
Astragalus onobrychis	3581	301.4	99.1	12.13	0.07
Astragalus declinatus	7338	349.1	101.2	33.07	0.17
Astragalus lineatus var. *lineatus*	1236	70.0	68.5	4.41	0.00
Astragalus oocephalus subsp. *stachyophorus*	200	44.5	74.9	0.51	0.00
Astragalus cinereus	935	99.8	107.3	27.42	0.00
Average	**2436**	**153.7**	**84.5**	**14.08**	**0.10**

Manganese (Mn) helps the formation of chloroplast together with Iron. In its deficiency, the chloroplast is disrupted and yellow spots are observed on the leaf blade. It is effective in the formation of chloroplastic protein. It acts as an enzyme and co-enzyme. Manganese is found in plant sections that are physiologically active, particularly the stem and shoots (Hawkes et al. 1985). The range of Mn in *Astragalus oocephalus* subsp. *stachyophorus* varied between 69.34 mg kg^{-1} in initial stage of vegetation and 115.91 mg kg^{-1} in seed forming stage. Optimal levels of manganese in plants are 25-250 mg kg^{-1} (Fox and Guerinot, 1998). Sheded et al. reported that the range of Mn in their research was between 44.6 and 339 mg kg^{-1} in selected medicinal plant of Egypt (Sheded et al., 2006). In this study with *Astragalus* taxa Mn ratio was detected an average (153.7 mg kg^{-1}) (*Table 3*).

Copper (Cu) assists in the formation of hemoglobin in humans, and it is required for disease resistance, seed production, and water regulation in plants. Despite the fact that Cu is a required element, its high concentration can cause anemia, stomach and intestinal discomfort, and liver and kidney damage (Martinez and Motto, 2000). The ratio of studied *Astragalus* taxa are seen in Table 3. In a research the Cu ratio of *Astragalus auganus* is 0.8234 mg kg^{-1} (Hussain et al., 2019). The concentration of Zinc (Zn) ranged from 0.51-34.22 mg kg^{-1} (except *Astragalus compactus*) and the average ratio was 14.08 mg kg^{-1} (*Table 3*). Hussain et al. (2019) reported the Zn concentration in *Astragalus auganus* as 2.750 mg kg^{-1}. When Beryllium (Be) levels increase in plants, growth decreases, Ca, Mg and partially P uptake are inhibited, and some proteins and enzymes are degraded (Kabata-Pendias, 2011). Beryllium (Be) was found in very small quantities or not found in studied taxa (*Table 3*).

Motsara and Roy (2008) stated that the optimum Fe content should be between 50-250 µg g^{-1}, Mn content between 20-300 µg g^{-1}, Cu content between 5-20 µg g^{-1} and Zn content between 20-100 µg g^{-1} in plants. It was observed that Fe and Cu contents of *Astragalus* taxa were high, Mn contents were sufficient and Cu contents were low.

The trace element contents of the studied *Astragalus* taxa are shown in *Table 4*. Nickel ratios of taxa varied between 6.24-19.66 mg kg^{-1}; and average Ni ratio is 9.99 mg kg^{-1}. *Astragalus declinatus* has highest Ni ratio (19.66 mg kg^{-1}). The lowest Ni rate is seen in *Astragalus lineatus* var. *lineatus* (4.18 mg kg^{-1}) (*Table 4*).

In the studied plants, there is no concentration of Cd (*Table 4*); therefore all studied *Astragalus* taxa of the area as for our findings are safe to be used in different aims. In a study conducted in Çanakkale, it was reported that the Cd content of pasture grasses was at a harmless level (Gokkus et al., 2013). Se ratios average of studied taxa is 2.19 mg kg^{-1}; whereas there is not found Se in *Astragalus lineatus* var. *lineatus* (*Table 4*). The concentrations of Al and Cr elements are seen in detailed in Table 4.

Kabata-Pendias (2011) stated that the optimum Al content should be between 85-3470 mg kg^{-1}, Cr content between 0.2-4.2 mg kg^{-1}, Ni content between 1.2-2.7 mg kg^{-1}, Se content for USA 0.32 mg kg^{-1}, for India 0.67 mg

kg^{-1} and Cd content between 0.8-0.46 mg kg^{-1} for legume plants. It was determined that Al, Ni and Se contents of Astragalus taxa were higher than the limit values reported by Kabata-Pedias (2011).

Table 4. Heavy metal contents of Astragalus taxa (mg kg^{-1})

Plant samples	Al	Cr	Ni	Se	Cd
Astragalus gummifer	1128	4.00	6.24	3.77	0.00
Astragalus compactus	2573	6.84	8.25	4.49	0.00
Astragalus lineatus var. *longidens*	2244	9.00	8.67	0.00	0.00
Astragalus aureus	3092	7.66	11.60	2.16	0.00
Astragalus onobrychis	3847	10.91	10.78	3.07	0.00
Astragalus declinatus	7685	17.42	19.66	0.90	0.00
Astragalus lineatus var. *lineatus*	1218	4.70	4.18	1.25	0.00
Astragalus oocephalus subsp. *stachyophorus*	205	1.98	9.55	1.75	0.00
Astragalus cinereus	820	6.55	10.96	2.31	0.00
Average	**2535**	**7.67**	**9.99**	**2.19**	**0.00**

4. Conclusion

In this study, 9 different *Astragalus* species were compared in terms of macro, micro element and heavy metal contents. As a result of the research, it was observed that the macro, micro element and heavy metal contents of some *Astragalus* species were higher than some forage plants. In general, the highest Ca, Mg, Fe, Mn, Al, Cr and Ni contents were observed in *Astragalus declinatus* species, the highest P and K contents in *Astragalus oocephalus* subsp. *stachyophorus* species, the highest Cu content in *Astragalus cinereus* species, the highest Zn content in *Astragalus lineatus* var. *longidens* species, the highest Be content in *Astragalus gummifer* species, the highest Se content in *Astragalus compactus* species. Cd content could not be detected in any *Astragalus* species. It has been concluded that *Astragalus* species, which are abundant in natural areas, can be a cheap source of roughage in terms of animal nutrition and can provide various macro and micro nutrients to animals.

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