

INVESTIGATION OF *ALKANNA ORIENTALIS* VAR. *ORIENTALIS* (L.) BOISS IN TERMS OF MACRO AND MICRO NUTRIENT ELEMENTS: A CASE STUDY IN AKSARAY

AYNUR DEMİR^{1*}, BULENT ESKİN¹ AND ADELEH RASHIDI²

¹Department of Urbanization and Environmental Problems, Aksaray University, Turkey

²Department of Environmental Engineering, Hacettepe University, Turkey

*Corresponding author's email: aynurdemir@aksaray.edu.tr; Ph: +90 (0382)2882505

Abstract

In this study, *Alkanna orientalis* var. *orientalis* (L.) BOISS was analyzed to determine its nutritional contents and phytoremediation potential. The plant and soil samples were collected from Mahmutlu Hill in Aksaray in May (2019). The mineral contents of soil and plant samples (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Ni, P, S, Zn) were determined by ICP-MS, and the obtained data were statistically analyzed and evaluated. K value in the soil was below the optimal value whereas Mg, Mn and Ni values were above the optimal values. On the other hand, the concentrations of Al, Ca, Cu, K, Mn and Zn in the plant were around the optimum values, while Fe, Mg, Ni were above. The results of the analysis showed that Fe and Ni concentrations were at a toxic level. Therefore this plant can be used in phytoremediation studies.

Key words: *Alkanna*, *Alkanna orientalis* var. *orientalis* (L.) BOISS, Autecology, Mineral nutrition, Plant-soil interaction.

Introduction

Alkanna orientalis (L.) Boiss. var. *orientalis* which belongs to the genus *Alkanna* is a significant member of the Boraginaceae family (Davis *et al.*, 1988). As a common taxon with golden colour and rarely bright yellow flowers, *A. orientalis* can grow up to 1 m in diameter and is a perennial plant that usually blooms in its second year. Basal leaves are lanceolate to oblong, and margin leaves are erose-undulate. Cauline leaves are oblong to ovate. Its leaves and flowers are covered with sticky, irritant, glands (Davis *et al.*, 1988; Wolff *et al.*, 1997). The plant flowers from the end of March until the beginning of August and produces approximately 10-60 flowers per plant. Its flowers are 1-2 cm long, golden-yellow and trumpet-

shaped. Four seeds can be produced per fruit on the plant (Wolff *et al.*, 1997; Güner *et al.*, 2012). The root of the species is taproot in shape and the pale-brown hard bark is present (Moustafa & Mansour, 2020).

A. orientalis is a widely common species that grows globally in South Greece, Syria, Lebanon, Palestine, Sinai, Transcaucasia, North Iran, and Turkey. The general distribution areas of the species in Turkey are northwest, southwest, and continental Anatolia (Fig. 1) (Aktaş, 2012; Anon., 2021). Rocky areas, steppes and volcanic slopes are among the habitats of *A. orientalis* which can live at an altitude of 0-2450 meters above sea level (Wolff *et al.*, 1997). It is an element of Irano-Turanian phytogeographic region (Gilbert *et al.*, 1996; Akçin *et al.*, 2004).



Fig. 1. Map of the study area (location of Mahmutlu Hill region, Aksaray-Turkey).

Although the species grows in many areas in Turkey, its natural breeding area is narrow. In addition, its breeding rate is fairly low. Therefore, this species is threatened by overgrazing, destruction of pasture areas, climate change, and over collection (Yaman *et al.*, 2019). Considering the recent negative effects of climate change, it is fair to state that the importance of studies on natural habitats and ecology of species is increasing.

In this context, *A. orientalis* is also an important plant species both ecologically and economically. In general, the studies on *A. orientalis* are focused on its usage in various areas. *A. orientalis* is widely known for its medicinal and pharmaceutical properties, depending on the chemical constituents of its plant parts. The most important part of the plant is the bark of the root which contains dye substances, and it has been used for dyeing purposes in textile, food, and cosmetics industries (Wafaa *et al.*, 2007; Abdel-Gelil *et al.*, 2019; Zannou & Koca, 2020; Moustafa & Mansour, 2020). It has been determined that these substances have various biological activities such as antimicrobial, anti-inflammatory, anticancer, anti-tumor, antioxidant, and enzyme inhibiting futures that are beneficial in medicine and pharmacology (Esfahani *et al.*, 2012; Petrosyan *et al.*, 2015; Onal *et al.*, 2017; Han *et al.*, 2019; Xu *et al.*, 2019; Yaman *et al.*, 2020). Additionally, the plant is known to be used in folk medicine to treat ulcus cruris and to heal wounds (Papageorgiou *et al.*, 2008). In addition, since the flowers of *A. orientalis* produce abundant nectar, they are the major source of many pollinators. For example, *Anthophora pauperata* is regarded as the main pollinator of *A. orientalis* (Gilbert *et al.*, 1996; Wolff *et al.*, 1997). As it can be seen, the studies on *A. orientalis* are mostly focused on medicinal properties. However, it is striking that the research on its ecological characteristics are limited. Evaluated from this point of view, the present study is of great importance for literature, and it is assumed that it would fill an important deficiency thanks to its results.

Therefore, the determination of ecological characteristics of species, on the one hand, gives information about the resources (such as micro-macro nutrients) that a living thing needs during its life cycle and, on the other hand, contributes to the creation of data on the subjects such as bioclimatic, soil and biotic characteristics of that region.

The importance of micro-elements in plant nutrition is great, and they should not be neglected in spite of their requirement minor quantities. The most important factors effecting plant growth and metabolism in natural habitats are macro and micronutrients. Among the macronutrients, nitrogen (N), phosphorus (P), and potassium (K) are the major nutrients, while calcium (Ca), magnesium (Mg), and sulphur (S) are the nutrients of secondary importance. The essential micronutrients are iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), molybdenum (Mo), chloride (Cl), and nickel (Ni). Sodium (Na), silicon (Si), and cobalt (Co) are also beneficial microelements. Micronutrients which are needed only in trace amounts are Fe, Mn, B, Zn, Cu, Mo, Cl, and Na (Bolat & Kara, 2017; Seven *et al.*, 2018). These essential micronutrients are also heavy metals and accumulate in the soil in various ways such as industrial waste and sewage disposal (Yılmaz *et al.*, 2020; Ghori *et al.*, 2019; Öztürk *et al.*, 2017a; Öztürk *et al.*, 2017b). Although some of these metals are essential micronutrients responsible for many

regular processes in plants, their excess, can have detrimental effects and directly influence the plant growth, metabolism, physiology, and senescence (Ghori *et al.*, 2019; Ozturk *et al.*, 2017a; Ozturk *et al.*, 2017b). In this regard, *A. orientalis* was investigated in this study in terms of micro and macro elements (Al, Ba, C, Ca, Cu, Fe, K, Mg, Mn, Ni, P, S, and Zn) considering the plant-soil interaction. In addition, the possible uses in phytoremediation are investigated in the study.

Materials and Methods

Description of the sampling sites: *A. orientalis* var. *orientalis* was collected from wild flora in Aksaray province Turkey (Fig. 1). These species were acquired at 1380 m attitude in May (2019) during the flowering period (Fig. 2). The study area in Mahmutlu Hill is a tectonically very active region, and Hasan Mountain and Karataş volcanism are also located in the area. The soil of the area is alluvial and lime-free brown (Uygur & Erkul, 2015). pH of the study area is 8.10 and alkaline. However, the bioclimatic level of this location is semi-arid Mediterranean climate with very cold winters (Akman, 2011). The meteorological data for Aksaray during the period between 1929 and 2020 shows, that the mean annual temperature is 12.1°C. In addition, the annual average precipitation is 362.3 mm (Anon., 2021). It indicates that the evaporation is quite high in the region and effects the soil structure (Demir *et al.*, 2021).

Preparation of the soil and plant samples: The samples of the plant and soil for the mineral element analyses were taken from four different locations in each sampling site. The soil sampling (about 500 g for each) was done using a stainless steel shovel, and the samples were taken from a depth of 15 cm. Soil samples were dried at room temperature or in a drying cabinet with air circulation not exceeding 40°C. Dried soils were pounded in a mortar or milled into friable clods. Then, stones, roots, and other foreign substances were cleaned by hand. Next, the soil samples were sifted through a 2 mm sieve, transferred to a suitable container, labelled, and finally used for analysis. A 0.25 mm sieve was used to prepare the soil sample less than 2 grams. Amounts between 0.04 and 50 mg of nitrogen, 0.02 and 175 mg of carbon, and 0.01 and 20 mg of sulphur were tested to determine the operating amount of CNS device.

Data analysis

In this study, Thermo Scientific X-Series II ICP-MS device, Thermo Scientific D 100 auto-diluter, and Cetac Asx-260 auto sampler accessories were used for the heavy metal measurements. A dilution of ultra-pure water with 5% nitric acid was used in all the samples. A High Purity Standards brand QCS-27 series calibration solution with 11 elements was used in the preparation of the calibration curves. The element concentration in the sample was taken into account, and the correlation coefficient was considered to be more than 0.99 when preparing the calibration curves of all the elements. For the analysis of the parameters, the main run number was taken as 3, and sampling and washing durations were set to be 60 seconds considering tubing length. The calibrations and the blank solution readings were repeated after every 40 readings.



Fig. 2. The photos were taken from Mahmutlu Hill in May showing a- Natural habitat of *Alkanna orientalis* var. *orientalis*, b- Flowers c- Aboveground parts of the plant (Eskin, 2019)

TruSpec Micro device was used as the elemental analyzer (CNS). TruSpec Micro delivers optimal performance in carbon, nitrogen, and sulfur determination in solid micro samples. The efficiency and speed of TruSpec Micro CNS is the result of the combination of a flow-through carrier gas system used in conjunction with individual, highly selective, infrared (IR) and thermal conductivity detection systems. Weighed micro samples (soil or plant) are placed into the autoloader of TruSpec Micro and are automatically dropped into the high-temperature (950°C) for carbon nitrogen and (1350°C) for S combustion furnace, allowing the sample to combust.

Statistical analyses

Multivariate analysis of variance (MANOVA) with Tukey's post-hoc HSD and Pearson correlation were calculated using IBM SPSS Statistics 20 software. The statistical significance was shown as $**P < 0.01$ and $*P < 0.05$ level (2-tailed).

Results and Discussion

The mean concentrations of heavy metal and mineral element distributions in the plant and soil samples of *A. orientalis* are presented in Table 1. In plant parts, element concentrations (mg/kg) are 2300-4000 for Al, 63-151 for Ba, 220000-370000, for C, 21000-34000 for Ca, 11-13 for

Cu, 2600-4500 for Fe, 17000-31000 for K, 3000-4000 for Mg, 129-255 for Mn, 18-27 for Ni, 1900-3600 for P, 25600-28000 for S and 22-36 for Zn. The average concentrations for Al, Ba, Ca, Cu, Fe, Mg, Mn and Ni elements ranked as flower < leaf < stem < root.

It means that *A. orientalis* intakes these elements through its roots in various ways (diffusion, endocytosis or through metal transporter) and uses them in metabolic activities. However, the average concentrations for C, K, P, and S elements ranked as flower > leaf > stem > root. Since P is effective in active metabolism, it is more abundant in flowers and leaves. K is an element that improves plant quality and also provides resistance to stress factors such as salinity and alkalinity in adverse conditions such as frost and drought (Öztürk *et al.*, 2017a; Öztürk *et al.*, 2017b). These values show the importance of these nutrients for the plant.

It is a known fact that one of the most important properties of the soil for the plant is the soil pH. The most suitable pH for plants to take up P and K is 6.5-7.5. pH for *Alkanna* is 8.10, and it is alkaline. This is one of the reasons why P, C, S and K ratios in *A. orientalis* structure are quite high. P and K concentrations were found to be high in the studies on *Alkanna haussknechtii* (Kandemir & Cansaran, 2010), *Pistacia lentiscus* (Aydın *et al.*, 2003) and *Centaurea hermannii* (Eroğlu, 2010). The results of the present study are consistent with literature.

Table 1. Heavy metal and mineral element distributions plant and soil samples.

Elements	mg/kg				*Optimum value in soil	*Optimum value in plant
	Flower	Stem/Leaf	Root	Soil		
Al	2300 ± 100	3000 ± 200	4000 ± 100	10000 ± 0.02/18000 ± 0.1	10000-40000	7-3470
Ba	63 ± 1	72 ± 1	151 ± 3	54 ± 1/30 ± 2	-	-
C	370000	300000	220000	64700/70000	-	-
Ca	21000 ± 1000	27000 ± 1000	34000 ± 1000	220000 ± 20000/140000 ± 10000	50000-150000	2000-30000
Cu	11 ± 1	11 ± 3	13 ± 1	15 ± 1/17 ± 2	5-30	5-30
Fe	2600 ± 100	3100 ± 200	4500 ± 100	7000 ± 200/5100 ± 100	5000-50000	50-250
K	31000 ± 1000	24000 ± 100	17000 ± 700	2500 ± 200/1900 ± 100	12000	1000-50000
Mg	3000 ± 100	3300 ± 200	4000 ± 100	20000 ± 2000/12000 ± 1000	3000-8000	100-1000
Mn	129 ± 1	189 ± 2	255 ± 1	755±11/600 ± 1	270-525	30-300
Ni	18 ± 1	19 ± 1	27 ± 1	19 ± 2/10 ± 1	10-50	0.1-5
P	3600 ± 100	2700 ± 100	1900 ± 100	300 ± 20	-	-
S	28000	26300	25600	22100/23800	-	-
Zn	36 ± 1	32 ± 2	22 ± 1	16 ± 2/11 ± 1	10-300	20-150

* References for Optimum Value in Soil and Plant (Pawlisz *et al.*, 1997; Kabata-Pendias & Pendias, 2001; Kabata-Pendias & Mukherjee, 2007; Kacar & Katkat, 2010)

The average values in the soil samples for Al, Ba, C, Ca, Cu, Fe, K, Mg, Mn, Ni, P, S, and Zn were 10000-18000, 300000-540000, 64700-70000, 14000-22000, 15-17, 5100-7000, 1900-2500, 12000-26000-755, 10-19, 300, 22100-23800, and 11-16, respectively (Table 1). The optimum value references in the soil (mg/kg) were found to be 50000-150000 for Ca, 5-30 for Cu, 5000-50000 for Fe, 12000 for K, 3000-8000 for Mg, 270-525 for Mn, 10-50 for Ni, and 10-300 for Zn (Pawlisz *et al.*, 1997; Kabata-Pendias & Pendias, 2001; Kabata-Pendias & Mukherjee, 2007; Kacar & Katkat, 2010). In this study, the concentrations of Cu, Fe, Ni and Zn in soil were within the optimal values. In addition, K was below the optimal value whereas Ca, Mg, and Mn were above the optimal values. K ratio in the flower of the plant is approximately 4 times more than that in the soil, and P ratio is approximately 12 times more than that in the soil. Accordingly, it can be concluded that *A. orientalis* generally prefers soil with deficiency in K but high concentration in Ca, Mg, and Mn.

The optimum value references of the mineral nutrients (mg/kg) in the plant have been measured as 7-3470 for Al, 2000-30000 for Ca, 5-30 for Cu, 50-250 for Fe, 1000-50000 for K, 100-1000 for Mg, 30-300 for Mn, 0.1-5 for Ni, and 20-150 for Zn (Pawlisz *et al.*, 1997; Kabata-Pendias & Pendias, 2001; Kabata-Pendias & Mukherjee, 2007; Kacar & Katkat, 2010). In this instance, the concentrations of Al, Cu, K, Mn and Zn in the plant are among the optimum values while Ca, Fe, Mg, Ni were above. It can be explained with the fact that the plant takes these minerals up from the soil and accumulates them. Additionally, the evaporation in the region is also effective in the accumulation.

Evaporation is particularly intense in July and August and fairly low between March and May in the study location. In the latter period, excessive rainfall and humidity cause higher macro and micro nutrient availability in the soil (Demir *et al.*, 2021). Due to the climatic feature of the region, Ca, and Mg values are quite high both in the plant and in the soil. The reason why these nutrients are high in the plant is that the plant accumulates them by taking them up during the low evaporation period. On the other hand, plants need sufficient Mg and Ca ions for their nutrition. However, plants can easily adapt to the scarcity or abundance of these elements.

Major macronutrients such as Ca, Fe, and Mg play important roles in plant growth and metabolism. Plants intake them from the soil in dissolved form (Bolat & Kara, 2017; Seven *et al.*, 2018). The fact that these nutrients are high in the soil makes it easier for the plant to supply these required nutrients. Fe is among the minor elements in soil and plants. It is taken up by plants in small amounts and used for the formation of chlorophyll (Seven *et al.*, 2018). Although the amount of Mn is high in the soil, it is not absorbed by *A. orientalis*. The high amount of Fe in the plant may have inhibited Mn absorption (Bosgelmez *et al.*, 2001).

According to the results of the analyses, Ni is quite dense in the plant. The amount of nickel in natural areas is generally very low. However, Ni is found in high amounts

in soil composed of serpentines. Ni, which passes into water with heavy rainfall, is removed from soil with leachate. In arid regions, Ni in the soil cannot be washed and therefore accumulates. Ni is required by the plant during the germination phase of the seed. In addition, Ni forms the metal part of urease, which is a catalase enzyme that converts urea to ammonium and carbon dioxide, and many hydrogenase enzymes. Therefore, it is an important source of micronutrients for plant metabolism (Kacar & Katkat, 2010). In this regard, it can be said that *A. orientalis* is a good Ni accumulator. It takes Ni through its roots and carries it to organs such as stems and leaves, where it can be easily removed. Ni is unstable and easily converted to oxides, carbonates, and sulphates (Yang *et al.*, 2000; Demir *et al.*, 2021). Minerals such as Ni, Mn, Fe, and Ca, which have become mobile, return to the upper layers with increased evaporation in summer. Therefore, it is predicted that Ni accumulation could be higher by *A. orientalis*. Therefore, it can be said that the plant has potential phytoremediation use for Fe and Ni.

Moreover, the correlation coefficients of the obtained element concentrations in the soil, root and leaf samples were examined to provide having insights about plant-soil interaction and element accumulation trends. It was determined that there was a high positive correlation between the root and stem/leaf (>0.99, >0.83) of the plant in terms of Al, Ba, Ca, Cu, K, Mg, Mn, P, S, and Zn nutrients in the correlation matrix (Table 2). It means that these nutrients are absorbed more in the root and stem of the plant than the other parts. In addition, the correlation between Al, Ba, Ca, Fe, K, Mg, Ni, and S in the root and Al, Fe, K, Mg, Mn, Ni, P and S in the stem/leaf (>0.58, >0.33) is low. In this case, these nutrients are stored less in the plant.

Table 3 shows the correlation matrix between the parts of the plant and the soil. It is noteworthy that there is a high positive correlation between the soil and plant parts in terms of Al, Ba, C, Ca, Cu, K, Mg, Mn, P, S, and Zn nutrients. It is understood that the correlation between Al,

Ca, Fe, Mg, Ni, and S in the soil and Ba in the stems/leaves is low (>0.38, >0.14). Likewise, the correlation between Al, Ba, Mg, Ni, and S in the soil and C in the flower (>0.58, >0.15) and the correlation between Al, Ba, C, Fe, and Ni in the soil and Ca in the root are also low (>0.62, >0.35).

According to the results of the correlation analysis, the nutrients in the soil and the plant are significantly correlated with each other displaying a positive relation between the plant and the soil in terms of nutrients. The relationship between the plant and the soil in which it grows is an important ecological factor. Depending on the development phase of the plant, the ratios of the elements it contains also vary and effect the entire vegetation period of the plant.

Conclusion

The presence of micro and macro elements depends on the interactions between soil and plant tissues. Some interactions cause interference on availability or damage to plant tissues due to excessive concentrations (Ozyigit *et al.*, 2013). Our investigations revealed that Fe, Mg, and Ni levels in our plant were more excessive than normal limits. In this regard, future research can be carried out on the effects of these minerals on plant development besides the ecology of *A. orientalis*.

The phytoremediation and ecological properties of *A. orientalis* have been investigated in this study. The present findings, along with the previous reports, indicate that the plant has a narrow propagation area, represents different accumulations for heavy metals and mineral nutrient elements, and prefers different soil properties. Additionally, *A. orientalis* differs from other studied plants for its habitat, element uptake, and soil preferences. Therefore, this work will provide future insights for plants living in narrow propagation areas and other newly identified species.

Table 2. Correlation matrix of mineral nutrients and heavy metals between root and stem/leaf samples.

Stem / Leaf	Root												
	Al	Ba	C	Ca	Cu	Fe	K	Mg	Mn	Ni	P	Zn	S
			0.823*	0.871	0.912*	0.930*	0.898*	0.878	0.834	0.814	0.952*	0.807	0.587
Ba	0.949**	0.837*	0.908*	0.981*	0.937*	0.865	0.943*	0.936*	0.845	0.922*	0.908*	0.881*	0.703
C	0.863	0.896**	0.975*	0.829	0.944*	0.957**	0.952*	0.960**	0.986**	0.971**	0.928*	0.990**	0.898*
Ca	0.987**	0.936*	0.562	0.943*	0.995**	0.969**	0.994**	0.9910**	0.948*	0.697*	0.987**	0.955*	0.773
Cu	0.843	0.797**	0.932*	0.973**	0.926*	0.938*	0.951*	0.9510*	0.956*	0.739*	0.605*	0.986**	0.951*
Fe	0.573	0.995**	0.957*	0.886*	0.648	0.631*	0.658	0.654	0.985**	0.975**	0.935*	0.992**	0.905*
K	0.935*	0.498*	0.654	0.969**	0.931*	0.938*	0.939*	0.951*	0.807	0.969**	0.907*	0.882*	0.906*
Mg	0.849	0.586	0.956*	0.971*	0.933*	0.948*	0.351	0.948*	0.889**	0.574*	0.919*	0.808*	0.871
Mn	0.760	0.854*	0.886*	0.584	0.867	0.885*	0.899*	0.901*	0.920*	0.940*	0.838	0.960**	0.974**
Ni	0.452	0.996**	0.975**	0.746	0.568	0.333	0.937*	0.767**	0.993**	0.687	0.910*	0.985**	0.867
P	0.889*	0.465	0.789**	0.949*	0.961**	0.958*	0.965**	0.953*	0.970*	0.849*	0.940*	0.996**	0.877
Zn	0.836	0.756*	0.960**	0.605*	0.815	0.838	0.862	0.856	0.955*	0.905*	0.784	0.989**	0.912*
S	0.605	0.905*	0.828	0.973*	0.827*	0.917*	0.940*	0.534*	0.860	0.978**	0.892*	0.922*	0.996**

*Correlation is significant at level of 0.05 (2-tailed), **Correlation is significant at level of 0.01 (2-tailed)

Table 3. Correlation matrix of mineral nutrients and heavy metals between soil and root, stem/leaf and flower samples.

	Soil												
	Al	Ba	C	Ca	Cu	Fe	K	Mg	Mn	Ni	P	Zn	S
Al Root	0.999**	0.815	0.998**	0.997*	0.999**	0.985*	0.995	0.986*	0.745	0.997*	0.997*	0.910	0.992
Al Stem/Leaf	0.998*	0.953	0.993*	0.982*	0.993*	0.998*	0.978	0.998*	0.817	0.999*	0.982	0.952	0.982
Al Flower	0.940	0.982	0.951	0.971	0.951	0.884	0.967	0.886	0.504	0.925	0.971	0.738	0.954
Ba Root	0.994*	0.957*	0.997*	0.998**	0.997*	0.969	0.998*	0.976	0.693	0.988	0.998**	0.887*	0.993
Ba Stem/Leaf	0.284	0.931*	0.916*	0.142	0.916	0.381	0.923*	0.374	0.812	0.290	0.908*	0.603*	0.321
Ba Flower	0.934*	0.995*	0.922*	0.891	0.922	0.975	0.882	0.851	0.944	0.949	0.891	0.999*	0.835
C Root	0.968*	0.866	0.976**	0.987*	0.976*	0.824	0.992*	0.926	0.583	0.956*	0.989*	0.799*	0.998**
C Stem/Leaf	0.998**	0.951	0.966**	0.997*	0.998**	0.981	0.995	0.986	0.745	0.997*	0.997*	0.915*	0.982*
C Flower	0.153	0.419	0.823*	0.521	0.994*	0.957	0.803	0.392	0.755	0.202	0.905	0.522	0.588
Ca Root	0.384	0.620	0.351	0.985*	0.956	0.511	0.926**	0.507	0.888	0.426	0.981*	0.710	0.769
Ca Stem/Leaf	0.961*	0.998**	0.646	0.938*	0.953	0.591	0.921**	0.912	0.911	0.974	0.928*	0.992	0.881
Ca Flower	0.997*	0.917*	0.991	0.999*	0.994	0.962	0.998**	0.963	0.672	0.983	0.999*	0.863	0.996
Cu Root	0.998**	0.953	0.999**	0.997*	0.998**	0.985*	0.995*	0.986*	0.745	0.997*	0.997*	0.910*	0.982*
Cu Stem/Leaf	0.974*	0.998*	0.996**	0.945*	0.966*	0.996*	0.938*	0.995*	0.891	0.983	0.944	0.986*	0.902*
Cu Flower	0.990**	0.907*	0.994*	0.996*	0.995**	0.962*	0.998**	0.953	0.672	0.983	0.996*	0.861*	0.996*
Fe Root	0.998*	0.843	0.998**	0.997*	0.998**	0.981*	0.986	0.909	0.548	0.997*	0.997*	0.773*	0.997*
Fe Stem/Leaf	0.999**	0.954	0.997*	0.992*	0.997*	0.992*	0.995	0.986	0.754	0.998*	0.992*	0.910**	0.982*
Fe Flower	0.957*	0.967	0.966	0.982	0.966	0.902*	0.989	0.993	0.778	0.943	0.982	0.930**	0.973*
K Root	0.963	0.998**	0.953	0.928*	0.953	0.991	0.921*	0.990	0.911*	0.974	0.928	0.992*	0.881
K Stem/Leaf	0.998*	0.951	0.998**	0.997*	0.999**	0.985	0.985	0.980	0.745	0.997*	0.997*	0.914*	0.982
K Flower	0.958	0.928	0.997*	0.998**	0.997*	0.969	0.998*	0.979	0.691	0.988	0.998**	0.877*	0.993
Mg Root	0.958	0.987*	0.993	0.928*	0.993	0.998*	0.978	0.998*	0.817	0.997*	0.982*	0.952**	0.954
Mg Stem/Leaf	0.996*	0.907	0.991	0.998*	0.991	0.965*	0.998*	0.958	0.654	0.979*	0.999**	0.851**	0.998
Mg Flower	0.987	0.998**	0.948	0.922	0.948	0.944	0.914	0.988	0.918	0.970*	0.922*	0.994**	0.954
Mn Root	0.968	0.866	0.976	0.989*	0.976	0.924	0.992	0.925	0.583*	0.956	0.989	0.981**	0.999**
Mn Stem/Leaf	0.974	0.998*	0.966	0.944	0.966	0.996	0.938	0.995	0.891*	0.853	0.944	0.986**	0.902*
Mn Flower	0.890	0.979*	0.874	0.835	0.874	0.921	0.824	0.941	0.972**	0.909	0.835	0.996**	0.769
Ni Root	0.989*	0.997*	0.973*	0.953	0.973*	0.998*	0.947	0.998*	0.877*	0.988*	0.953	0.981*	0.914
Ni Stem/Leaf	0.987*	0.907*	0.991*	0.998*	0.991*	0.955*	0.998*	0.956	0.651	0.979*	0.998*	0.852*	0.998*
Ni Flower	0.994**	0.928*	0.997*	0.998**	0.997*	0.960	0.999*	0.970*	0.693	0.941*	0.842	0.877*	0.993*
P Root	0.994*	0.928	0.997*	0.998**	0.997*	0.969	0.997*	0.971	0.541	0.988	0.998**	0.847	0.951
P Stem/Leaf	0.998*	0.944	0.998*	0.989*	0.999*	0.979	0.996*	0.989	0.726	0.994	0.997*	0.898	0.987
P Flower	0.958	0.846	0.967	0.983	0.967	0.909	0.984	0.911	0.552	0.945	0.983	0.777	0.997*
Zn Root	0.996**	0.982	0.993	0.982	0.993	0.998*	0.978	0.998*	0.817	0.998*	0.982	0.952**	0.954*
Zn Stem/Leaf	0.929*	0.992	0.910	0.877	0.997	0.968	0.861	0.966	0.951*	0.939	0.877	0.998**	0.967*
Zn Flower	0.980*	0.891	0.982	0.995	0.986	0.943	0.997*	0.941	0.625	0.970	0.995	0.832**	0.998*
S Root	0.891*	0.981	0.876	0.838	0.874	0.946	0.824	0.944	0.974	0.910*	0.832	0.997*	0.972*
S Stem/Leaf	0.998*	0.973	0.997	0.989	0.997*	0.994	0.986	0.995	0.781	0.998**	0.989	0.934	0.967*
S Flower	0.952	0.835	0.961	0.978	0.961*	0.901	0.983	0.903	0.535	0.938	0.974	0.763	0.982*

* Correlation is significant at level of 0.05 (2-tailed), ** Correlation is significant at level of 0.01 (2-tailed).

References

- Abdel-Gelil, O.E., N.A. Atwa, A.R.A. Moustafa and S.R. Mansour. 2019. *Alkanna* species: a promising herbal medicine and its uses. *J. Food Sci. & Nutr. Res.*, 2(4): 309-315.
- Akçin, Ö.E., N. Kandemir and A. Cansaran. 2004. A morphological and anatomical study on endemic *Alkanna haussknechtii* Bornm. (Boraginaceae), critically endangered in Turkey. *Turk. J. Bot.*, 28(6): 591-598.
- Akman, Y. 2011. *Climate and Bioclimate*. Palme Publication Distribution, Ankara, Turkey.
- Aktaş, K. 2012. *Alkanna*. Bizimbitkiler <<http://www.bizimbitkiler.org.tr>>, Acces Date: 29.09.2021.
- Anonymous. 2021. The official website of Turkish State Meteorological Service, <http://www.MGM.gov.tr>. Acces Date: 28.04.2021.
- Anonymous. 2021. Turkish Plants Data Service. *Alkanna orientalis* (L.) Boiss. var. *orientalis* (L.) Boiss. <http://www.tubives.com/> Acces Date: 30.05.2021.
- Aydın, H., S. Baslar, Y. Doğan and H.H. Mert. 2003. A study of soil-plant interactions of *Pistacia lentiscus* L. distributed in the western Anatolian part of Turkey. *Acta Bot. Croatica*, 62(2): 73-88.
- Bolat, I. and Ö. Kara. 2017. Plant nutrients: sources, functions, deficiencies and redundancy. *J. Bartın Facul. Forest.*, 19(1): 218-228.
- Bosgelmez, A., İ.İ. Bosgelmez, S. Savasçı and N. Pashı. 2001. *Ecology – II (Soil)*, Baskent Klise Press., Kızılay-Ankara, Turkey.
- Davis, P.H., R.R. Mill and K. Tan. 1988. *Flora of Turkey and the East Aegean Islands*. Vol: 6. Edinburgh University Press, Edinburgh.

- Demir, A., G.B. Furtana, M. Tekşen and R. Tıprıdamaz. 2021. Determination of the heavy metal contents and the benefit/cost analysis of *Hypericum salsugineum* in the salt lake vicinity. *Sains Malasia*, 50(12): 1-12.
- Eroğlu, H.K. 2010. *Autecological Characteristics of Centaurea hermannii F.Hermann (Asteraceae), An Endemic Species for Istanbul*. Marmara University Graduate School of Natural and Applied Sciences. Department of Biology, Master Thesis, Turkey.
- Esfahani, H.M., Z.N. Esfahani, N.K. Dehaghi, A. Hosseini-Sharifabad, K. Tabrizian, M. Parsa and S.N. Ostad. 2012. Anti-inflammatory and anti-nociceptive effects of the ethanolic extracts of *Alkanna frigida* and *Alkanna orientalis*. *J. Nat. Med.*, 66(3): 447-452.
- Ghori, N.H., T. Ghori, M.Q. Hayat, S.R. Imadi, A. Gul, V. Altay and M. Ozturk. 2019. Heavy metal stress and responses in plants. *Int. J. Environ. Sci. & Technol.*, 16(3): 1807-1828.
- Gilbert, F., P. Willmer, F. Semida, J. Ghazoul and S. Zalut. 1996. Spatial variation in selection in a plant-pollinator system in the wadis of Sinai, Egypt. *Oecologia*, 108(3): 479-487.
- Güner, A., S. Aslan, T. Ekim, M. Vural and T. Babaç. 2012. *Turkey Plants List*. Nezahat Gökuyigit Botanical Garden and Flora Research Association Publication, İstanbul/Turkey.
- Han, X., K.A. Kang, M.J. Piao, A.X. Zhen, Y.J. Hyun, H.M. Kim, Y.S. Ryu and J.W. Hyun. 2019. Shikonin exerts cytotoxic effects in human colon cancers by inducing apoptotic cell death via the endoplasmic reticulum and mitochondria-mediated pathways. *Biomol. & Therapeut.*, 27(1): 41-47.
- Kabata-Pendias, A. and A.B. Mukherjee. 2007. *Trace Elements from Soil to Human*. Springer Science & Business Media. New York, USA.
- Kabata-Pendias, A. and H. Pendias. 2001. *Trace Elements in Soils and Plants*, CRC Press., Boca-Raton, USA.
- Kacar, B. and V. Katkat. 2010. *Plant Nutrition*. 5th Edition, Nobel Publication, Kızılay-Ankara, Turkey.
- Kandemir, N. and A. Cansaran. 2010. An autecological investigation on endemic *Alkanna haussknechtii* Bornm. (Boraginaceae) critically endangered in Turkey. *Res. J. Agri. & Biol. Sci.*, 6(5): 613-618.
- Moustafa, A.A. and S.R. Mansour. 2020. Impact of climate change on the Distribution behavior of *Alkanna orientalis* in Saint Catherine, south Sinai, Egypt. *Catrina. The Int. J. Environ. Sci.*, 22(1): 29-34.
- Onal, A., F. Eser and S. Bayrak. 2017. Investigation of dyeing properties of different fabric species with *Alkanna orientalis* root extract. *J. Food Sci. & Eng.*, 7: 213-220.
- Ozturk, M., I. Uysal, E. Yücel, V. Altay and E. Karabacak. 2017a. Soil-plant interactions in the monumental plane trees (*Platanus orientalis*) grove-Çanakkale-Turkey. *J. Environ. Biol.*, 38(5): 1129-1137.
- Ozturk, M., V. Altay and F. Karahan. 2017b. Studies on trace elements in *Glycyrrhiza* taxa distributed in Hatay-Turkey. *Int. J. Plant & Environ.*, 3(2): 01-07.
- Ozyigit, I.I., I. Dogan, B. Eskin, M. Keskin, G. Demir and I.E. Yalcin. 2013. Mineral element uptake status of endemic *Isoetes anatolica* Prada & Roller populations from Bolu-Turkey. *Pak. J. Bot.*, 45(1): 515-519.
- Papageorgiou, V.P., A.N. Assimopoulou and A.C. Ballis. 2008. Alkannins and shikonins: a new class of wound healing agents. *Curr. Med. Chem.*, 15(30): 3248-3267.
- Pawlisz, A.V., R.A. Kent, U.A. Schneider and C. Jefferson. 1997. Canadian water quality guidelines for chromium. *Environ. Toxicol. & Water Quality. An Int. J.*, 12(2): 123-183.
- Petrosyan, M., Y. Shcherbakova, N. Sahakyan, Z. Vardanyan, A. Poladyan, Y. Popov and A. Trchounian. 2015. *Alkanna orientalis* (L.) Boiss. plant isolated cultures and antimicrobial activity of their extracts: phenomenon, dependence on different factors and effects on some membrane-associated properties of bacteria. *Plant Cell, Tissue & Organ Culture*, 122(3): 727-738.
- Seven, T., B. Can, N.B. Darende and S. Ocağ. 2018. Heavy metals pollution in air and soil. *Nat. Environ. Sci. Res. J.*, 1(2): 91-103.
- Uygur, A. and S.K. Erkul. 2015. Flora of area between the villages of Karaoren-Yuva-Karkın (Aksaray-Turkey). *Bagbahce Sci. J.*, 1(3): 39-62.
- Wafaa, A.T., A.S. Khaled, S.A. Nahla, M.H. Nahed and I.I. Shams. 2007. Naphthaquinones of *Alkanna orientalis* (L.) Boiss. *Afr. J. Trad., Compl. & Altern. Med.*, 4(1): 55-58.
- Wolff, K., S. El-Akkad and R.J. Abbott. 1997. Population substructure in *Alkanna orientalis* (Boraginaceae) in the Sinai desert, in relation to its pollinator behaviour. *Mol. Ecol.*, 6(4): 365-372.
- Xu, J., K. Koizumi, M. Liu, Y. Mizuno, M. Suzaki, H. Iitsuka, A. Inujima, M. Fujimoto, N. Shibahara and Y. Shimada. 2019. Shikonin induces an anti-tumor effect on murine mammary cancer via p38-dependent apoptosis. *Oncol. Reports*, 41(3): 2020-2026.
- Yaman, C., S. Uranbey, E. R. Muhammet and D. Başalma. 2020. Secondary metabolite contents and antioxidant activities of some *Alkanna* taxa under *In vivo* and *In vitro* conditions. *Turk. J. Agri. & Nat. Sci.*, 7(3): 618-626.
- Yaman, C., S. Uranbey, H.A. Ahmed, S. Özcan, O. Tugay and D. Başalma. 2019. Callus induction and regeneration of *Alkanna orientalis* var. *orientalis* and *A. sieheana*. *Bangladesh J. Bot.*, 48(3): 633-640.
- Yang, Y.Y., J.Y. Jung, W.Y. Song, H.S. Suh and Y. Lee. 2000. Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. *Plant Physiol.*, 124: 1019-1026.
- Yılmaz, Ş.E., Ş. Öztürk and B. Aslım. 2020. Effects of some heavy metals on thiol compounds in *Synechocystis* sp. isolates. *Int. J. Environ. Sci. & Technol.*, 17: 3013-3022.
- Zannou, O. and I. Koca. 2020. Optimization and stabilization of the antioxidant properties from Alkanet (*Alkanna tinctoria*) with natural deep eutectic solvents. *Arab. J. Chem.*, 13(8): 6437-6450.