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Determination of Major and Minor Elements in the *Malva sylvestris* L. from Turkey Using ICP-OES Techniques

Ü. Hiçsönmez · F. S. Ereeş · C. Özdemir · A. Özdemir · S. Çam

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Abstract In this work, *Malva sylvestris* var. *mauritiana* (L.) leaves were collected from different points in Muradiye region of Manisa-Turkey. The leaves were dissolved by wet digestion method using a mixture of mineral acid. Concentrations of Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, La, Mg, Mn, Na, Ni, Pb, Sn, Sr, Sb, Si, Ti, U, Zn, and Zr in prepared solutions were determined by using inductively coupled plasma optical emission spectrometry (ICP-OES). High Ca (13,848 mg/kg) and Mg (1,936 mg/kg) concentrations were found at the leaves. Obtained values were compared with the internationally permitted (standard) values. The results of elements were analyzed statistically (analysis of variance test). For different leaf sizes, concentration factors were calculated.

Keywords ANOVA · *Malva sylvestris* · Element analysis · ICP- OES

Introduction

Traditional approach to study plant chemistry is mainly focused on plant mineral nutrition and toxicity of certain elements. Environmental aspects of plant chemistry as a research area have gained considerable interest during the last 20–30 years [1–6].

Various elements are transmitted into living metabolism through food intake. Although some of these elements are beneficial for the human body, some of them may be toxic or radioactive. ^{40}K, ^{238}U, ^{235}U, or the radioactive daughters in the U and Th natural decay

Ü. Hiçsönmez (✉)
Department of Chemistry, Faculty of Sciences and Arts, Celal Bayar University, Manisa, Turkey
e-mail: umran.hicsonmez@bayar.edu.tr

F. S. Ereeş · S. Çam
Department of Physics, Faculty of Sciences and Arts, Celal Bayar University, Manisa, Turkey

C. Özdemir
Department of Biology, Faculty of Sciences and Arts, Celal Bayar University, Manisa, Turkey

A. Özdemir
Department of Mathematics, Faculty of Sciences and Arts, Celal Bayar University, Manisa, Turkey

chains may be taken via consumption of these elements. In particular, these radioactive elements may cause internal radiation risk [7, 8].

Manisa region located in the Western part of Turkey has very productive agricultural soil. The *Malva sylvestris* L. is herbaceous being consumed by human and animals in the region. *M. sylvestris* L. is a biennial–perennial herbaceous plant distributed in Europe and North Asia [9]. There are 6–8% musilage in the leaf of the plant. Glucose, Arabinoz and Rawnoz are obtained by hydrolysis of the plant musilage [10]. *M. sylvestris* is used in traditional phytotheropy [11] and cosmetic treatments [12]. Fluid extract of *M. sylvestris* flower and leaves are used as a valuable remedy for cough and inflammatory diseases of mucous membranes [13]. Sulfite oxidase was isolated from *M. sylvestris* L. [14]. In addition, the plant has laxative and protective effect and is used as an abortive in rural locations [15].

Due to the importance of mineral and trace elements present in medical herbs, several studies have been carried out to determine their concentration levels by using atomic absorption spectrometry (AAS), inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), neutron activation analysis, X-ray fluorescence, and electrochemical methods [16–21]. Good detection limits, a large linear dynamic range, relative freedom from chemical inter-element interferences, simultaneous multielement capability make ICP-OES a powerful analytical tool for many applications [3].

Many analytical methods to determine the trace element in plant material require decomposition of the sample. Sample preparation is still the major factor contributing to the uncertainty of the final results. This is especially important in the determination of trace elements in environmental studies [22]. ICP-OES generally requires sample presentation as a liquid. This involves the destruction of organic material by wet or dry oxidation. Mixtures such as HNO_3 + H_2O_2, HNO_3 + $HClO_4$, and HNO_3 were already proposed for sample solubilization for multielement ICP-AES analysis many years ago [23].

This study compares 26 chemical elements analyzed in *M. sylvestris* var. *mauritiana* (L.) species collected over university campus area in Muradiye-Manisa, Turkey. Samples dissolved with two different methods. Analysis of variance (ANOVA) test applied for leaf size and two dissolving methods. The chemical composition of these plants can have important implications for animal and ultimately human health.

Materials and Methods

Sample Preparation and Measurement

All acids and other reagents used were of analytical grade purity (Merck and Riedel-de Halen). Deionized water, obtained by passing water through pure water system (innovation Human Power 1 Scholar), was used to prepare solutions and all samples.

Manisa is located in the cenetr of the region in the west coast of Turkey, called Aegean, with a latitude of 38.38 N and longitude of 27.30 E (http://www.mapsofworld.com/lat_long/turkey-lat-long.html). Leaves of *M. slyvestris* have been collected from five different points of university area in Muradiye-Manisa (Turkey). Collected samples were separated into three different sizes, e.g., smaller than 3.3 cm as small, between 3.3 cm and 5.4 cm as medium, and bigger than 5.4 cm as large.

Collected samples were washed and then dried at 50 °C for 7 h in an incubator. Samples were digested via two different methods.

Method I

One gram of powdered dry samples were weighed into 100 mL glass beakers. Three milliliters of 65% HNO_3 and 5 mL of 35% H_2O_2 was added to the samples and allowed to react overnight. The following morning, the beakers were carefully heated until clear solutions were obtained. Samples were carefully studied without letting them dry. A mixture of 3 mL 65% HNO_3 and 9 mL 37% HCl was added and gently heated until a small volume of acid remained. The residue was filtered, and solutions were precisely transferred to 100 mL plastic standard flasks and made to volume with deionized water.

Method II

One gram of the plant sample was heated with 12 mL 37% HCl and 4 mL 65% HNO_3 acid mixture in a glass beaker. The mixture was evaporated until it dried. The residue was digested by mixing 65% HNO_3 and 37% HCl (1:3) again and filtered. The filtered acid extract was carefully transferred to 100 mL plastic standard flasks, and a volume was made with deionized water.

The element concentrations in all samples were measured by using a Perkin-Elmer 2001 Model inductively coupled plasma-optic emission spectrometry (ICP-OES).

Results and Discussion

Analytical results for all analyzed elements in each of 15 *M. sylvestris* L., which dissolved with Methods I and II, were given in Tables 1 and 2, respectively. Major elements (median >1,000 mg/kg) are the same in all samples: Ca and Mg, followed by K and Fe (see Table 1). Ag, Cd, La, Ni, Sb, and Zr were not detected in all samples.

There is little reliable information about the content of Al in human and animal food. The content in fruit and vegetables varies with a range of 0.1 to 5.0 mg/kg. According to the available data, rye and wheat contain approximately 5.0 mg/kg of Al, and rice about 1.5 mg/kg [24]. Concentrations of 1,211±86 mg/kg and 611±13 mg/kg were determined in tobacco leaves and tomato leaves, respectively [19]. Approximately 4.0 mg Al per kg fresh material is found in mushrooms [24]. The content of aluminum ranged between 6.4 and 158 mg/kg in our study.

Barium (Ba) is not considered an essential element for plants [25]. Soluble Ba salts are quite toxic for animals. Ba leaf concentrations among the samples range from 3.4 to 19.1 mg/kg. Geochemically, Ba resembles Ca and Sr. The considerable difference in Ba leaf concentrations between the plants [pine (2.7 mg/kg) and birch (81.4 mg/kg) at the extreme ends] is not a direct reflection of the difference in the Ca concentrations, although pine shows also the lowest and birch the highest Ca concentration [5].

Boron (B) is an essential element for higher plants, and boron deficiency is much more widespread than toxicity [25]. It appears that it plays a role in calcium utilization and in the development of the actively growing parts of the plant [5]. Reimann et al. reported that birch and blueberry show the highest B concentrations, and it is the lowest concentration for moss. Jones (1972) gave a value of 15 mg/kg for a large variety of crops as the critical level of B in plants below which deficiency can be expected, while toxicity occurs at levels above 100 mg/kg [26]. Table 1 demonstrates that B concentrations in the samples generally change between 0.1 and 0.5 mg/kg. However, sample IV (big) prepared with Method I is detected as 2.3 mg/kg.

Table 1 Concentration of Major and Trace Elements Dissolved with Method I in the Study of *Malva sylvestris* L. Samples (mg/kg as Dry Weight)

Element	Sample-I (mg/kg)			Sample-II (mg/kg)			Sample-III (mg/kg)			Sample-IV (mg/kg)			Sample-V (mg/kg)		
	Small	Med.	Big	Small	Med.	Big	Small	Med.	Big	Small	Med.	Big	Small	Med.	Big
Ag	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Al	158	70.4	64.4	32.5	55.3	27.1	17.9	47.7	12.3	6.4	15.9	17.2	23.3	25.2	36.6
B	0.1	0.1	0.2	0.2	0.5	0.4	0.2	0.4	0.4	0.2	0.4	2.3	0.3	0.4	0.4
Ba	9.1	13.2	13.5	8.2	11.1	12.5	3.4	15.6	5.8	14.7	19.1	15.1	7.8	9.0	7.4
Bi	ND	0.5	0.6	0.8	1.0	0.6	0.6	0.8	0.7	0.9	0.4	0.8	0.7	0.5	0.5
Ca	11,800	10,910	12,920	9,199	15,970	19,000	6,778	15,650	11,120	14,490	18,300	17,750	16,540	18,840	15,700
Cd	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Co	1.2	1.1	1.0	1.1	1.0	0.9	1.1	1.1	1.1	0.9	1.0	0.9	1.0	1.0	1.0
Cr	0.4	0.2	0.3	0.2	0.2	0.2	3.9	0.5	0.2	0.7	0.2	0.1	0.2	0.2	0.2
Cu	8.7	9.2	8.7	9.8	9.0	8.8	9.4	8.8	10.3	6.1	6.5	5.6	9.5	8.0	7.8
Fe	106.3	92.3	84.2	64	95	78.2	68.2	102.5	40.7	62.1	58.7	57.1	75.6	94.6	73.2
K	367.3	301.0	371.3	311.4	340.4	357.4	265.7	212.6	224.1	237.0	290.1	220.1	271.3	299.3	283.0
La	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND	ND	ND
Mg	2,195	2,071	2,246	1,833	2,071	2,298	1,308	1,894	1,928	1,971	1,362	1,914	1,860	1,799	1,752
Mn	24.2	22.1	22	21.9	28.5	28	18.9	23.3	18.8	22.2	25.4	17.4	27.7	34.6	27.0
Na	46.6	45.7	34.7	22.4	83.1	48.2	ND	26.7	31.8	26.7	15.8	5.2	ND	ND	ND
Ni	ND	ND	ND	ND	ND	ND	0.7	ND	ND	ND	ND	ND	ND	ND	ND
Pb	1.5	1.8	2.0	1.5	1.7	1.4	1.2	1.4	1.3	1.2	1.3	1.1	1.7	1.5	1.6
Sb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Si	36.1	33.9	36.4	27.3	33.8	32.3	23.6	12.4	16.3	30.3	36.2	32.6	27.5	15.4	14.3
Sn	1.9	1.2	1.6	3.5	10.1	10	5.4	2.1	1.4	13.5	15.1	12.9	15.5	15.4	15.7
Sr	30.5	38.0	45.2	33.3	53.8	61.9	15.9	47.2	28.3	46	65.3	62.2	29.5	45.7	33.8
Ti	0.9	0.7	0.6	0.4	1.4	0.6	0.4	0.2	ND	0.4	0.6	0.4	1.2	0.5	0.7
U	0.6	0.5	0.5	0.4	0.4	0.5	0.5	0.1	0.5	0.1	0.5	0.4	0.5	0.3	ND
Zn	18.8	15.9	14.1	18.0	14.7	12.4	23.8	17.9	12.3	19.5	17.6	16.2	25.5	33.4	23.1
Zr	ND	ND	ND	ND	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] *ND* not detected

Table 2 Concentration of Major and Trace Elements Dissolved with Method II in the Study *M. sylvestris* var. *mauritiana* Samples (mg/kg as Dry Weight)

Element	Sample-I (mg/kg)			Sample-II (mg/kg)		
	Small	Medium	Big	Small	Medium	Big
Ag	ND	ND	ND	ND	ND	ND
Al	28.7	13.3	34.2	39.3	61.0	31.0
B	ND	ND	0.1	0.8	4.5	ND
Ba	6.5	6.7	11.3	5.9	5.3	4.3
Bi	ND	ND	ND	0.3	0.9	1.0
Ca	8,234	6,457	9,539	10,980	10,860	6,760
Cd	ND	ND	ND	ND	ND	ND
Co	0.7	1.2	1.1	1.4	1.4	1.4
Cr	0.2	0.02	0.2	0.2	0.2	0.1
Cu	9.1	5.3	9.7	9.5	9.2	6.5
Fe	92.3	56.1	105.7	56.2	58.9	42.5
K	1,800	5,000	653.8	133.4	112	96.8
La	ND	ND	ND	ND	ND	ND
Mg	1,096	664.9	1,717	1,292	1,421	1,056
Mn	17.6	10.7	19.9	18.9	20.2	12.4
Na	36.0	26.1	50.7	ND	ND	ND
Ni	ND	ND	ND	ND	ND	ND
Pb	ND	1.5	2.4	3.5	1.9	2.1
Sb	0.2	0.2	0.3	0.1	ND	0.3
Si	68.6	23.3	84.0	71.8	174.6	71.8
Sn	ND	ND	ND	2.3	1.5	1.1
Sr	26.0	21.5	33.0	34.9	29.9	20.3
Ti	1.8	0.9	1.8	0.7	1.0	0.3
U	ND	0.3	0.003	0.4	0.5	0.8
Zr	ND	ND	0.001	0.1	0.1	0.1
Zn	8.5	4.0	10.2	13.2	13.1	13.4

ND not detected

Calcium is an essential element which is found at high concentrations in plants [24, 27]. It was found to be 14.9 mg/g in apple leaves [3]. All the studied samples showed high Ca concentrations.

Cobalt and nickel are considered to be essential for man, plants, and animals. Cobalt is a component of vitamin B12 [21, 24]. Although this element is required only in small quantities for maintaining normal health, lower or higher concentrations may lead to toxicity or deficiency resulting in impairment or abnormalities [21]. However, the natural levels of this element in biological materials still remain unknown. In addition, cobalt (Co) is an element that is specifically required for nitrogen fixation and for plant growth [25]. Also, Co was found 0.1–0.2 mg/kg by Adeloju in a performed work [21]. But, Co concentration in our work was found between 0.9 and 1.2 mg/kg. These values remain the same in all samples regardless of area and size.

Copper (Cu) is an important micronutrient, but also quite toxic at higher concentrations [5]. Jones reported that 5–20 mg/kg in plant tissue is necessary for normal growth, while less than 4 mg/kg is considered as deficient, and more than 20 mg/kg is already in the toxic range [26]. Sheded et al. reported that both *Pergularia tomentosa* and *Cympobogon proximus* plants in Egypt accumulated the highest amount of copper (27.0 and 22.0 mg/kg, respectively), while the accumulation of copper ranged between 6.3 mg/kg in *Balantes aegyptiaca* and 8.2 mg/kg in *Acaia ehrenbergiana* [6]. Reddy and Reddy reported that the range of Cu contents in 50 medicinally important leafy materials growing in India were 17.6–57.3 mg/kg [28]. In this work, minimum Cu concentration found to be as 5.6, whereas maximum was found to be 10.3 mg/kg.

Sheded et al. reported that the concentration of Fe in Hegleeg (*Balanites aegyptiaca*), Halfa bair (*Cympobogan proximus*), and Ghalga (*Pergularia tomentosa*), which are widely use in Egypt were 261, 492, and 1,239 mg/kg, respectively [6]. Suggested Fe requirement for animals ranges between 30 and 100 mg/kg and the maximum tolerable level for cattle is 1,000 mg/kg [29–31]. The highest Fe concentration in examined sample was to be 106.3 mg/kg, while the lowest was found to be 40.7 mg/kg.

Lead (Pb), toxic for plants, ranges from 1.1 to 2.0 mg/kg in this study. Reimenn et al. reported that a high Pb concentration (4.13 mg/kg) is observed for moss [5].

There is recent evidence suggesting that magnesium may play a major role in coordination with metabolism control and growth in animal cells. Magnesium is an essential element, and the effects of its deficiency in plants, animals, and humans are known with typical symptoms. Mg is widespread in all living cells (the second most common intracellular iron after potassium). Marine algae contain 6 to 20 g/kg, whereas plants contain 1 to 8 g/kg [24]. Mg is seen as the second main element in the studied samples.

Manganese is an essential element for plant growth, though it is required in small quantity. It is also an important element from the point of view of biochemical activity, since it associates with an antioxidant enzyme superoxide dismutase (Mn-SOD). Kulkarni et al. showed that Mn concentration increases with plant growth period in both shoots and roots [19]. Reddy and Reddy reported that the range of Mn in their study was 10.5–81.6 mg/kg. In this work, it was observed that there was no correlation between plant size and Mn content [28]. The range of manganese in the examined plants was found to be 17.4–34.6 mg/kg.

Potassium is one of the most important macronutrients essential for the plant growth [24]. Also, K concentration was found to be higher compared to other elements studied except for Ca, Mg (Table 1).

Role of sodium is still not clearly understood in plant physiology, but there are a few evidences suggesting that Na might be a beneficial micronutrient. Though its concentration is only 14.7±0.4 mg/kg in the seed, higher quantities are found in shoots, and even higher quantities were found in roots [19]. Sheded et al. found that the range of sodium was high with a minimum of 1.22% in *Acacia ehrenbergiana* and a maximum of 3.45% in *Citrulluscolocynthis* [6]. But, Na concentrations were not found at high level in our leaves.

Sr concentration also shows relative differences from size to size. Sr value is generally higher in the medium and large leaves than in small leaves.

Wrenn, et al. reported that ranges of the measured U concentrations in the foods analyzed were 0.3–30 ng U/g wet weight for plant products (fruit, vegetables, and grains, both raw and prepared) and 0.0005–4 ng U/g wet weight for animal products (meat, fish, poultry, eggs, milk, both raw, and prepared) [32]. Almost in all plants, U concentration is less than 0.005 mg/kg. But on the contrary, 0.029 mg/kg was found in moss [5]. Uranium concentration was found to be between 0.1 and 0.6 mg/kg as shown in Table 1.

Zinc (Zn) is an important micronutrient. It is known that plants vary widely in their optimum requirement for Zn [25]. There may be large differences at the Zn concentrations found in different plant leaves. For example, while Zn concentration in birch was found to be 205 mg/kg, in willow, it was found to be 125 mg/kg, whereas it was 14 mg/kg in blueberry. The concentration of zinc in tulsi (*Ocimun sanctum*) and neem (*Azadirachtaindica*)

Table 3 The Comparison of Element Concentrations Detected in the Big- and Small-sized Leaf (Method I)

Element	Mean		SD		*F* ratio	Probability	Significance
	c	*d*	*c*	*d*			
Al	42.90	47.62	22.22	62.42	0.03	0.877	NS[a]
B	0.360	0.200	0.151	0.070	4.57	0.065	NS
Ba	13.60	8.640	3.931	4.040	3.87	0.085	NS
Ca	15,936	11,761	3,139	3,927	3.45	0.100	NS
Cr	0.260	1.080	0.134	1.590	1.32	0.284	NS
Cu	8.300	8.700	1.105	1.508	0.23	0.645	NS
Fe	88.62	75.24	17.16	18.12	1.44	0.265	NS
K	288.66	290.54	46.72	50.45	0.00	0.953	NS
Mg	2,039	1,833	215.1	326.5	1.39	0.273	NS
Mn	26.78	22.98	5.001	3.248	2.03	0.192	NS
Pb	1.540	1.420	0.207	0.217	0.80	0.397	NS
Si	27.16	27.84	12.24	2.807	0.01	0.907	NS
Sr	31.38	31.38	8.59	8.59	0.66	0.439	NS
Ti	0.680	0.660	0.444	0.372	0.01	0.940	NS
Zn	19.90	2.03	7.658	0.192	0.11	0.752	NS

[a] *NS* Not significant

c big leaf, *d* Small leaf

*$P<0.05$

**$P<0.01$

leaves, which is widely used in Indian Ayurvedic medicine, was found as 140 and 10 mg/kg, respectively [33]. The maximum tolerable zinc level was as 500 mg/kg for cattle and 300 mg/kg for sheep [31]. There are various Zn concentration levels found in this study in the range of 12.3 and 33.4 mg/kg. There is no difference for Zn concentrations at different locations. In addition, concentrations do not depend on the size of leaves (Table 1).

Table 4 The Correlations Between Element Concentrations in Method I

Element	Mean	SD	*F* ratio	Probability	Significance
Al–B	47.62–0.20	62.42–0.07	2.89	0.128	NS[a]
Al–Ba	47.62–8.64	62.42 –4.04	1.94	0.201	NS
Al–Cr	47.62–1.08	62.42–1.59	2.78	0.134	NS
Al–Mn	47.62–22.98	62.42–3.25	0.78	0.404	NS
Al–Zn	47.62–21.12	62.42–3.32	0.90	0.371	NS
B–Ba	0.20–8.64	0.07–4.04	21.81	0.002	**
B–Cr	0.20–1.08	0.07–1.59	1.53	0.251	NS
Ba–Cr	8.64–1.08	4.04–1.59	15.16	0.005	**
Ba–Pb	8.64–1.42	4.04–0.22	15.92	0.004	**
Ba–Zn	8.64–21.12	4.04–3.32	28.47	0.001	**
Co–Al	1.08–47.62	62.42–0.11	2.78	0.134	NS
Co–Cu	1.08–8.640	4.040–0.110	17.49	0.003	**
Co–Pb	1.08–1.420	0.1095–0.217	9.80	0.014	**

[a] *NS* not significant

*$P<0.05$

**$P<0.01$

Table 5 The Comparison of Element Concentrations of Different Sized Leaf in the Between Two Locality (Method I)

Leaf	Mean		SD		*F* ratio	Probability	Significance
	e	*f*	*e*	*f*			
Big-sized	849	2,731	1,168	3,980	0.07	0.793	NS[a]
Middle-sized	989	1,370	3,230	4,736	0.07	0.792	NS
Small-sized	1,724	504	4,799	1,688	0.92	0.345	NS

[a] *NS*: Not significant

e locality 1, *f* locality 2

*$P<.05$

**$P<.01$

Results were statistically tested by using one-way analysis of variance (ANOVA) to determine the significant differences. While results of Method I statistically showed a meaning relation, results of Method II did not show. Results of the statistical analysis were presented in Table 3 through Table 6.

It was found that there were no statistically important differences between element concentrations and the leaf size (Table 3).

It was reported by Reimann et al. that moss showed good correlation of Al–Fe over the whole concentration range, and many other elements (e.g. Zr, As, Cr, Si, U) also displayed a high correlation with Al [5]. In our study, it was seen that Al has a relation with B, Co, Cr, Ba, Zn, and Mn (probability values: 0.1–0.4).

According to our study, there were statistically important relationships between Co–Cu, Co–Pb, Ba–B, Ba–Zn, Ba–Cr, and Ba–Pb at the significance level of $P<0.01$ (Table 4). But it was seen that many elements in the leaf did not show a meaningful relation at the significance levels of $P<0.05$ and $P<0.01$.

Statistical values of the results obtained by Methods I and II were presented in Tables 4 and 5, respectively. According to these results presented in Tables 5 and 6, Method I (H_2O_2, HNO_3, and HCl mixtures) gave statistically much meaningful results than Method II (HNO_3, HCl mixtures).

Table 6 The Comparison of Element Concentrations of Different Sized Leaf in the Between Two Locality (Method II)

Leaf	Mean		SD		*F* ratio	Probability	Significance
	e	*f*	*e*	*f*			
Big-sized	764	791	2,381	2,735	0.01	0.976	NS[a]
Middle-sized	767	798	1,962	2,706	0.01	0.970	NS
Small-sized	1,724	504	4,799	1,688	0.92	0.345	NS

[a] *NS* not significant

e locality 1, *f* locality 2

*$P<0.05$

**$P<0.01$

Conclusion

This present study is related to the major and trace element contents in the *Malva sylvestris*, which could potentially be either dangerous or useful for humans who are consuming medicinal plants or for animals feeding on this plant. There were no significant differences between big and small-sized leaves with respect to element concentrations in the studied leaves. The amount of Ca and Mg in all studied leaves was higher in concentrations than the other detected elements in leaves. The determined element concentrations were between the international safety limits both for human and animal consumption.

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