Frontiers in Life Sciences and Related Technologies 5(3) (2024) 182-187

Contents lists available at Dergipark

Frontiers in Life Sciences and Related Technologies

Journal homepage: http://dergipark.org.tr/en/pub/flsrt

Research article

Determination of heavy metals and trace element contents in *Veronica grisebachii* S. M. WALTERS

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Abstract

This study aimed to determine the concentrations of Al (aluminum), Ba (barium), Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), Mn (manganese), Ni (nickel), Pb (lead), S (sulphur), and Zn (zinc) in soil and *Veronica grisebachii* S. M. WALTERS samples. The research focused on the heavy metal and essential nutrition element contents of these plant species. Plant samples were collected from southeastern Aksaray province, Türkiye, at the geographical coordinates 38°13'54.5"N 34°08'28.8" E and an elevation of 1276 m above sea level. Standard methods were used to determine the plant (root, stem, and leaf parts) and soil elements. The numerical values of essential elements and heavy metals in the species were quantified using ICP-MS. XRF device was also used to determine the elements in the soil. The results showed that the amounts of Cr, Cu, Fe Pb, S, and Zn in the soil were within the optimum range, while the concentrations of Al, Co, Mn, and Ni were above the optimum values. The levels of Al, Co, Mn, and Ni in the soil of the plant's natural habitat were above the reference values. This species has a high capacity to absorb and accumulate heavy metals such as Al, Co, Mn, and Ni from the soil.

Keywords: *Heavy metals; ICP-MS; trace elements; Türkiye; Veronica*

1. Introduction

Veronica is a large genus belonging to the Plantaginaceae family, with approximately 500 species, generally distributed in Southern and Central Europe as well as Türkiye (Ozyigit et al., 2013; Salehi et al., 2019; Hai et al., 2024). Plantaginaceae includes 94 genera and 1900 species in the world (Yaylaci et al., 2018). It consists herbs, subshrubs or rarely small trees, with exstipulate, opposite, alternate or whorled leaves. The flowers are hermaphrodite, and are typically borne solitary in the leaf axils. Additionally, they can be found in racemose, spicate, or paniculate cymes (Oztunca, 2009).

Veronica grisebachii, called Kesan blue in Türkiye, is an annual plant. This plant (Fig. 1) has an upright stem and can grow from 4 cm to 20 cm (Ulukus and Tugay, 2008). Generally, its branches are abundant and densely pubescent. Leaves with 3-7 mm petiole, lower leaves ovate to elliptic, shallowly lobed, or entire. Its upper part is palmate or pinnatifid, with 3-5 lobes. The dense flowers are glandular and pubescent. Corolla bright to deep blue and 8-12 mm diam. Seeds are almost 4-angled, thick, rugulose, and yellowish (Davis et al., 1978).

V. grisebachii S. M. WALTERS is a species that can grow at altitudes of 700-1600 meters above sea level. Its distribution areas cover habitats specific to the Eastern Mediterranean phytogeographic region, including sparse maquis, *Pinus* forests, steppes, maquis, and meadows. Aksaray, Antalya, Ankara, Burdur, Bursa, Eskisehir, Edirne, Izmir, Karaman, Nevsehir, Sinop, Tekirdag and Usak are among the important distribution areas of this species in Türkiye (Turkish Plants Data Service, 2023).

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https://doi.org/10.51753/flsrt.1441355 Author contributions
Received 22 February 2024; Accepted 20 September 2024
Available online 30 December 2024

Fig. 1. A- General view of *Veronica grisebachii*, B- Close up view of the flower, C-Leaves of plant.

An examination of the existing research on *V. grisebachii* reveals a significant focus on floristic studies on the species. However, the research concerning its uptake of heavy metals and utilization of trace elements is notably limited. Moreover, the species' response to the pollution of heavy metals and its potential for phytoremediation is not yet fully understood. For this reason, this study's purpose is to research the heavy metal retention capacity and trace element utilization potential of *V. grisebachii*. In particular, metals such as Al, Ba, Co, Cr, Cu, Fe, Mn, Ni, Pb, S, and Zn were evaluated based on the interaction between soil and plants. These elements play a vital role both in determining the nutritional value of the soil and in the growth and development of the plant (Agac et al., 2024). During the uptake of these trace elements (Ba, Mn, and S) by plants, some heavy metals (Zn, Pb, Ni, Fe, Cu, Co, Cr, and Al) are also taken up and included in the food chain (Pugazhendhi et al., 2024). However, the excessive amounts of these heavy metals in the soil can cause environmental pollution and lead to toxic effects for both plants and other organisms that consume these plants (Ozyigit, 2021; Osma et al., 2023). In addition, heavy metals can disrupt the normal structure and function of cellular components, interfering with various metabolic and developmental processes. (Angon et al., 2024; Yazicioglu et al., 2024). Therefore, it is thought that determining the phytoremediation potential of this species will make a significant contribution to the literature both concerning combating heavy metal pollution and better understanding the ecological characteristics of the plant.

2. Materials and methods

2.1. Study area

Aksaray province (Fig. 2) is situated in the middle section of the Central Anatolia Region. The coordinates of Aksaray province are 8-39 N and 33-35 E. It borders Nevsehir, Nigde, Konya, Ankara, and Kirsehir. The province is also distinguished by the presence of Tuz Lake, Türkiye's second-largest lake, in its northwestern region (Doganay and Eskin, 2018; Kalkan and Terzi, 2023). According to the Population-Based Registration System Database, Aksaray has 433.055 population in a land area of 7.997 km^2 (Aksaray Governorship, 2024; Turkish Statistical Institute, 2024).

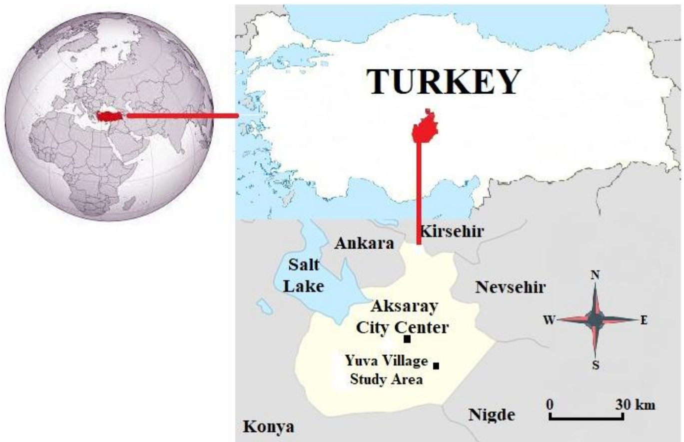

Fig. 2. Map of the research area (Location of Yuva Village in Aksaray, Türkiye).

2.2. Topography, soil and geology

The study site is located in a tectonic basin surrounded by mountains and a saline lake. The area exhibits a significant elevation difference, ranging from a low of 705 m to a high of 3,275 m. In addition, most of the Aksaray center and districts are covered with flat lands (Akyazi, 2019). Major soil groups of the research area include non-calcareous brown soils, brown soils, and colluvial soils (Uygur, 2014). Aksaray province is covered with lands formed by calcareous volcano tuffs formed in the tertiary. Stratigraphically, the metamorphics of the Kırşehir massif called the Kaman Group, are located at the bottom of the land. The metamorphites are overlain by gabbro and gabbronorite masses called Karakaya ultramaphite. Eocene and Oligocene aged cover units lie unconformably on these units (Kiraz, 2013).

2.3. Climate

Türkiye is climatically divided into regions with a Mediterranean climate and regions with a non-Mediterranean climate (Akman, 2011). The bioclimatic layer of Aksaray is the Mediterranean climate with semi-arid summers and very cold winters (Baser, 2014). In the study area, there is generally high temperature and very little precipitation in the summer months. The annual mean temperature was measured as 12.1°C (Table 1)

Table 1
Climate data for Aksaray (Between 1929-2022).

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Average highest Temperature (°C)	5.5	7.5	12.5	18.1	23.1	27.1	30.7	30.7	26.7	21	13.8	7.7	18.7
Average temperature (°C)	0.5	2.1	6.3	11.5	16.2	20.2	23.5	23.3	18.8	13.3	7.2	2.6	12.1
Average low (°C)	-3.6	-2.2	1.2	5.6	9.7	13.1	16.2	15.9	11.4	6.9	2	-1.4	6.2
Precipitation (mm)	40.7	35.1	40.9	45.2	43.6	29.3	7.1	5.4	12.2	23.4	31.5	46.2	361
Average precipitation days	9.97	9.97	10.6	10.5	10.4	6.22	1.54	1.47	2.79	5.5	7.09	10.5	86.5

in about the last hundred years. Summer weather is warm, with high temperatures in July and August averaging 30.7°C. (MGM, 2023). According to these calculated climate analyses, Aksaray province is among the driest areas in Türkiye (Donmez and Yildiz, 2023).

2.4. Collection, preparation, and analysis of samples

Samples of *V. grisebachii* were collected from Yuva Village Aksaray, Türkiye in May during the period of flower. One location in the same neighborhood was determined for heavy metal status and mineral nutrients. 10 plants were taken from this location because the plant was too small. Soil samples taken from under each plant were mixed and turned into a single sample. Samples were taken in the area where the plant is densely populated. In addition, approximately 500 grams of soil samples were taken from a depth of 0-15 cm with a shovel. The plant samples were dried in the oven at 80°C for 48 hours. Then, dried samples were ground with a micro hammer and passed through a 1.5 mm mesh sieve. In the next stage, 0.2 grams of samples were placed in Teflon containers, and 4 ml of 65% HNO_3, was added. These samples were mineralized using a microwave oven (CEM MARS 5) following at the three-step temperature level (145°C for 5 minutes, followed by 165°C for 5 minutes, and finally 175°C for 20 minutes). After mineralization and cooling, the samples were filtered using Whatman filters and then were diluted to a final volume of 50 ml using ultrapure water and transferred to Falcon tubes. By preparing a multi-element standard solution at -1000 ppm (Merck) from the stock solution, the quantitative values of mineral elements and heavy metals were determined via Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Thermo, Xseries 2 Serial number: SN02132C).

An ICP Multi-Element Standard solutions were created by diluting a 10 mg kg^{-1} stock solution to obtain final concentrations of 1, 5, 6, 10, 30, 50, 100, 150, and 200 µg kg^{-1}. Then, a calibration curve was created using equal concentrations of trace elements (Ahamad et al., 2017; Ozbay et al., 2023). In addition, numerical results for the same minerals in the soil were obtained by the XRF device (Pan Analytical, Axios Max Serial number: DY5970).

Numerical results for the same minerals in the soil were obtained by the XRF device (Pan Analytical, Axios Max Serial number: DY5970). In preparation stage, soil samples were ground to 20 µm particle size using a ball bearing tungsten carbide cap and a 5 g aliquot was homogenized with 1 g of Micropulver Wachs C. The mixture of wax and sample was pressed into pellets using a die attacher at 13 kg N^{-1}. The pressed pellet was analyzed with XRF to identify the heavy metals (Demir et al., 2021).

The $CaCO_3$ ratio in the soil was detected using a Scheibler calcimeter (Caglar, 1949). The soil pH was measured with a pH meter. Organic matters were measured according to Walkley-Black (Black, 1965). Additionally, the total phosphorus of the samples was determined with Olsen and Sommers methods (Olsen and Sommers, 1982). The photometric method was used to determine the amount of available potassium in the soil.

2.5. Statistical analyses

Pearson correlation coefficients were calculated for average and standard deviation values using SPSS (version 25). The significance level of the data was found to be statistically significant at $^{**}P<0.01$ and $^{*}P<0.05$ levels (2-tailed).

2.6. Bioconcentration factor

The Bioconcentration Factor (BCF) is a quantitative measure of the extent to which a plant species can take metals from the soil. It is determined by dividing the metal concentration in plant tissue by the corresponding concentration in the soil, based on the analysis of elements. Plants exhibiting a BCF value exceeding one (>1) are promising for extracting contaminants from soil through phytoremediation (Ghosh and Singh, 2005; Demir et al, 2021).

BCF = metal concentration in the plant tissue/metal concentration in the soil

3. Results and discussions

The mean numerical values of heavy metal and trace element distributions in soil and plant samples are shown in Table 2. The average values (mg kg^{-1}) for Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S, and Zn in the soil samples were 66900, 485.6, 100, 27, 25, 33400, 700, 51.5, 22.7, 24.8 and 60.3 respectively. The optimum reference values (mg kg^{-1}) in soil are as follows: Al: 10,000-40,000, Cr: 5-120, Co: 1-10, Cu: 5-30, Fe: 5,000-50,000, Mn: 270-525, Ni: 10-50, Pb: 10-30, S: 10-157, and Zn: 10-300 (Kabata-Pendias and Pendias, 2001; Barker and Pilbeam, 2007; Kabata-Pendias and Mukherjee, 2007; Kacar and Katkat, 2010; Blum et al., 2014). The results of the analysis showed that the concentrations of Cr, Cu, Fe, Pb, S, and Zn in the soil were within the optimum reference values. However, the values of Al, Co, Mn, and Ni were found to be above the optimum ranges. On the other hand, the element concentrations (mg kg^{-1}) in plant parts, without considering standard errors, were determined as follows: Al: 327.53-932.84, Ba: 18.54-84.01, Cr: 11.18-66, Co: 0.23-0.63, Cu: 3.71-5.15, Fe: 1009.2, Mn: 16.69-36.10, Ni: 0.74-3.80, Pb: 0.60-0.72, S: 0.55-1, and Zn: 19.21-20.72. Optimal reference values for mineral nutrient and heavy metal elements in plants (mg kg^{-1}) are as follows: Al: 7-3470, Cr: 0.1-0.5, Co: 0.02-0.5, Cu: 5-30, Fe: 5-250, Mn: 30-300, Ni: 0.1-5, Pb: 0.05-3, and Zn: 20-150 (Kabata-Pendias and Pendias, 2001; Jones and Jacobsen, 2005; Kabata-Pendias and Mukherjee, 2007; Blum et al., 2014).

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When the element concentrations in the plant parts were compared with the optimum values: It was observed that the concentrations of Al, Cu, Mn, Ni, Pb, and Zn complied with the reference values. This shows that these elements are at appropriate levels for plant growth and development. It was seen that the concentrations of Cr, Co, and Fe elements were above

Table 2
Mineral element and heavy metal concentration values in soil and plant samples.

Veronica grisebachii	Root	Stem and Leaf	Soil	Optimum Value in Soil	Optimum Value in Plant	Bioconcentration Factor (BCF)
Al (mg kg^{-1})	932.84	327.53	66900	10000-40000	7-3400	0.0139
Ba (mg kg^{-1})	18.54	84.01	485.6	-	-	0.0381
Cr (mg kg^{-1})	11.18	66	100	5-120	0.1-0.5	0.111
Co (mg kg^{-1})	0.63	0.23	27	1-10	0.02-0.5	0.0233
Cu (mg kg^{-1})	5.15	3.71	25	5-30	5-30	0.206
Fe (mg kg^{-1})	1009.2	247.28	33400	5000-50000	5-250	0.0302
Mn (mg kg^{-1})	36.10	16.69	700	270-525	30-300	0.0515
Ni (mg kg^{-1})	3.80	0.74	51.5	10-50	0.1-5	0.0737
Pb (mg kg^{-1})	0.72	0.60	22.7	10-30	0.05-3	3.1718
S (mg kg^{-1})	1	0.55	24.8	10-157	-	0.0403
Zn (mg kg^{-1})	19.21	20.72	60.3	10-300	20-150	0.3185

Refrences for bioconcentration factor (BCF) and optimum value in soil and plant (Kabata-Pendias and Pendias, 2001; Ghosh and Singh, 2005; Jones and Jacobsen, 2005; Barker and Pilbeam 2007; Kabata-Pendias and Mukherjee, 2007; Kacar and Katkat, 2010; Blum, Horak and Mentler, 2014)

the optimum values. This situation indicates that these minerals are taken up from the soil and stored by the plant. The fact that certain minerals are taken up from the soil and stored by the plant indicates that the plant can utilize these minerals for growth, development, and metabolic processes. The ion form of these minerals, taken up from the soil through *V. grisebachii* roots, can be stored in different tissues of the plant. This storage allows the plant to reserve the minerals it needs for growth periods or stress conditions. In addition, in *V. grisebachii*, values for Ba, Cr, and Zn are arranged as root < stem-leaf, while values for Al, Co, Cu, Fe, Mn, Ni, Pb, and S are arranged as root > stem-leaf. The concentrations of Ba, Cr, and Zn are the highest in the roots. The concentrations of Ba, Cr, and Zn are found at the highest levels in the roots, while the concentrations of Al, Co, Cu, Fe, Mn, Ni, Pb, and S are at the highest levels in the leaves of species. This situation shows that different elements are transported and stored differently within *V. grisebachii*. indicating that Ba, Cr, and Zn are needed more in the regions close to the roots, while Al, Co, Cu, Fe, Mn, Ni, Pb, and S are transported to the leaves for processes such as photosynthesis or other metabolic activities.

Also, Bioconcentration Factor (BCF) of the studied heavy metals varied 0,0139 for Al, 0,0381 for Ba, 0,111 for Cr, 0.0233 for Co, 0,206 for Cu, 0,0302 for Fe, 0,0515 for Mn, 0,0737 for Ni, 3,1718 for Pb, 0,0403 For S, 0,3185 for Zn (Table 2). The Pb ratio (3,1718) in this species is greater than 1 (>1). This shows that this plant may be a good hyperaccumulator of Pb.

Table 3 shows the physical analysis results of soil samples taken from the distribution area of *V. grisebachii*. When these results are evaluated, it can be observed that the natural habitat of *V. grisebachii* consists of clay-loamy and slightly alkaline soils with a pH of 7.89. The level of CaCO3 is low (1.25%), and the Saturation value is 0,028% (non-saline). In addition, the organic matter concentration of the soil was found to be of low (1.37 %) value. These findings indicate that *V. grisebachii* has a certain tolerance to soil type and chemical properties. Also, the result of correlation analysis between root and stem/leaves is a high level of positive correlation (>1.00, >0.82) between the root and shoot/leaf for the nutrient elements Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S, and Zn (Table 4). These nutrient elements indicate that they are transported and stored in a coordinated manner among different organs of the plant. In addition, the correlation between Al, Ba, Cr, Co, Cu, Pb in the root and Al, Ba, Cr, Co, Cu, Fe Mn Ni, and Zn in the stem/leaf (>0.58, >0.33) is low. This means that these elements are transported and stored differently between roots and stems/leaves.

Table 3
Physical analysis results of the soil samples of *Veronica grisebachii* habitats.

Analysis Type	Soil Numerical value	Status
Texture (%)	52.58	Clayey-loamy
$CaCO_3$ (%)	1.25	Low Chalky
pH	7.89	Slightly Alkaline
Saturation (%)	0,028	Without salt
Potassium (K_2O) kg/da	177.55	Adequate
Phosphorus (P_2O5) kg/da	1.63	Very Low
Organic Matter (%)	1.37	Low

According to the result of the correlation between soil and plant parts is a high level of positive correlation between soil and plant parts for the nutrients Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S, and Zn (Table 5). While it is understood that the correlation is low (>0,001, >0.59) in some soils and stems/leaves, it is high (>1, >0.61) in others. This indicates that the element concentrations in the soil directly affect the element concentrations stored in different organs of the plant.

It is possible for the elements found in high concentrations in the soil to be more absorbed and stored by the roots, stems, and leaves of the plant (Phuong et al., 2023). This shows that the element concentrations in the soil can directly affect plant nutrient uptake and growth. The plant-soil interaction is a critical ecological factor. As a plant grows, the amounts of different elements in it change, which affects its whole life cycle.

In conclusion, the close relationship between Veronica and its habitat shows that this plant has the potential to be used in phytoremediation studies.

4. Conclusion

The levels of Ni, Mn, Co, and Al in the soil of the plant's natural habitat are above the reference values. This shows that the soil is exposed to heavy metal pollution. In addition, *V. grisebachii* accumulates Cr, Co and Fe nutritional elements. According to BFC factor the Pb ratio in this species is greater than 1. This result shows that the plant can be a good Pb hyperaccumulator. This research results indicate that there is a strong relationship between nutrients in *V. grisebachii* and soil.

Table 4
Correlation relationship between mineral nutrients in root and stem/leaf.

	Correlation Matrix (R)										
Pearson Correlation	**Al Root**	**Ba Root**	**Cr Root**	**Co Root**	**Cu Root**	**Fe Root**	**Mn Root**	**Ni Root**	**Pb Root**	**S Root**	**Zn Root**
Al Stem/Leaf	0.012**	1.00**	0.051	0.866	0.008	0.5	0.668	0.688	0.027	0.561	0.507
Ba Stem/Leaf	0.967	0.996	0.030*	0.822	0.817	0.427	0.551	0.692	0.054*	0.596	0.453
Cr Stem/Leaf	0.027*	0.026*	0.233	0.566	0.686	0.455	0.996	0.985	0.998	0.955	0.829
Co Stem/Leaf	0.001	0.009**	0.511	0.5	0.406	0.211	0.219	0.98	0.999	0.758	0.844
Cu Stem/Leaf	0.008**	0.005**	0.998	0.492	0.994	0.908	0.861	0.968	0.994	0.995	0.336
Fe Stem/Leaf	0.064	0.063	0.993	0.443	0.24	0.973	0.24	0.241	0.242	0.16	0.638
Mn Stem/Leaf	0.996	0.996	0.028*	0.823	0.079	0.429	0.148	0.464	0.052*	0.445	0.498
Ni Stem/Leaf	0.052	0.051*	0.994	0.453	0.644	0.637	0.619	0.146	0.996	0.997	0.329
Pb Stem/Leaf	0.672	0.671	0.705	0.211	0.793	0.705	0.784	0.858	0.721	0.977	0.265
S Stem/Leaf	0.189	0.181	0.971	0.327	0.314	0.191	0.314	0.313	0.976	0.561	0.727
Zn Stem/Leaf	0.063	0.065	0.999	0.554	0.676	0.599	0.572	0.665	0.547	0.559	0.661

Table 5
Correlation relationship between mineral nutrients in soil and plant parts.

	Correlation Matrix (R)										
	Al Soil	**Ba Soil**	**Cr Soil**	**Co Soil**	**Cu Soil**	**Fe Soil**	**Mn Soil**	**Ni Soil**	**Pb Soil**	**S Soil**	**Zn Soil**
Al Root	0.012 **	1.000**	0.082	0.866	0.114	0.24	0.199	0.167	0.363	0.09	0.904
Al Stem/Leaf	0.012**	1.000**	0.082	0.866	0.114	0.24	0.199	0.167	0.363	0.996	0.904
Ba Root	0.013**	0.001**	0.081	0.866	0.113	0.241	0.202	0.168	0.363	0.091	0.905
Ba Stem/Leaf	0.069	0.996*	0.163	0.822	0.195	0.16	0.118	0.086	0.285	0.009**	0.866
Cr Root	0.999	0.054**	0.991	0.427	0.952	0.981	0.996	0.993	0.949	0.999	0.472
Cr Stem/Leaf	0.118	0.024**	0.998**	0.476	0.996	0.963	0.974	0.98	0.921	0.993	0.402
Co Root	0.51	0.8674	0.427	0.05*	0.397	0.693	0.662	0.637	0.78	0.576	0.996
Co Stem/Leaf	0.999	0.002**	0.405	0.337*	0.258	0.97	0.979	0.985	0.931	0.995	0.427
Cu Root	0.999	0.002**	0.996	0.468	0.993	0.651	0.979	0.461	0.931	0.995	0.427
Cu Stem/Leaf	0.999	0.005	0.322	0.427	0.994	0.968	0.984	0.984	0.928	0.995	0.419
Fe Root	0.872	0.502	0.822	0.866	0.803	0.996**	0.948	0.937	0.988	0.907	0.821
Fe Stem/Leaf	0.997	0.061	0.999	0.443	0.998	0.953	0.964	0.937	0.531	0.822	0.367
Mn Root	0.996	0.064	0.999	0.449	0.998	0.766	0.964	0.391	0.905	0.987	0.365
Mn Stem/Leaf	0.067	0.996	0.929	0.996	0.938	0.337	0.121	0.233	0.966	0.011**	0.867
Ni Root	0.977	0.194	0.993	0.319	0.996	0.985	0.921	0.933	0.841	0.958	0.24
Ni Stem/Leaf	0.333	0.049	0.052	0.997	0.052	0.051	0.05	0.978	0.911	0.989	0.379
Pb Root	0.360	0.028	0.994	0.523	0.989	0.977	0.985	0.990	**0.941**	0.998*	0.451
Pb Stem/Leaf	0.672	0.671	0.793	0.211	0.812	0.557	0.591	0.617	0.721	0.676	0.291
S Root	0.5	0.497	0.904	0.576	0.993	0.72	0.748	0.77	0.96	0.817	0.082
S Stem/Leaf	0.996	0.067	0.994	0.327	0.997	0.907	0.924	0.99	0.846	0.96	0.248
Zn Root	0.85	0.538	0.526	0.886	0.948	0.911	0.934	0.922	0.981	0.889	0.845
Zn Stem/Leaf	0.998	0.536	0.989	0.554	0.984	0.899	0.994	0.99	0.953	0.999	0.484

This research is the first study on the phytoremediation possibilities of *V. grisebachii* species. Therefore, it is thought that the results of this research will make a significant contribution to the literature.

Acknowledgement: The authors would like to thank the Aksaray University Scientific and Technological Application and Research Center for its contribution to the experimental analyses in the present study. In addition, they would like to thank botanist expert Dr. Seher Karaman for identifying *V. grisebachii* plant.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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Cite as: Rashidi, A., Eskin, B., & Demir, A. (2024). Determination of heavy metals and trace element contents in *Veronica grisebachii* S. M. WALTERS. *Front Life Sci RT*, *5*(3), 182-187.