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Trace element analysis in some *Salvia* species by inductively coupled plasma-mass spectrometry (ICP-MS) and chemometric approach

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ABSTRACT: In this study, the stems, leaves, flowers, roots and mixed all parts of five *Salvia* species were analyzed for their trace element (Li, Be, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Hg, Tl, Pb, and U) contents using ICP-MS. The seeds, roots leaves, flowers, and mixed parts of each species were digested by concentrated nitric acid and hydrogen peroxide in a microwave by before ICP-MS the analysis. The accuracy and precision of the method were evaluated by CRM 1573a Tomato Leaves. Trace element contents in different parts of each sample were compared. Concentration of toxic elements (As, Cd, Hg and Pb) were lower than those declared by WHO, except Cr content. Cr content in the root sections of *Salvia suffruticosa* (SFR), *S. hydrangea* (SHR), *S. trichoclada* (STR), *S. xanthocheila* (SXR), leaf samples of *S. kronenburgii* (SKL) and *S. xanthocheila* (SXL). also the Cr content in the leaf sections of *Salvia kronenburgii* (SKL) Salvia *xanthocheila* (SXL) was found high. When consider the daily metal (Zn, Cu, Sr, Ba and Ni) needs It was concluded that these S*alvia* species can be nutritive sources. In addition, *Salvia* samples were classified by utilizing chemometric techniques such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). While the first two main components explained 55.30% of the total variance, the first six main components explained 89.60% of total variance

KEYWORDS: *Salvia*, trace element, ICP-MS, PCA.

1. INTRODUCTION

Salvia genus a member of the Lamiaceae family grows naturally all over the world with more than 900 species. It is represented, by 89 species and 97 taxa, 45 of which are endemic in Turkish flora [1]. Many of the Lamiaceae species include essential oils which are used in perfumery and pharmaceutical industries [2]. Medicinal plants having biological activity have been used in the treatment of a variety of diseases since ancient times [3,4].

Many *Salvia* species have been used as tonic and spice and consumed as tea by the local people since ancient times. *Salvia* species have also been used in the treatment of various diseases [2]. There are many papers show that *Salvia* species, containing high amount of phenolic compounds, exhibit good antioxidant and antimicrobial activities. Moreover, some were also investigated against various pathological diseases such as atherosclerosis, cerebral function disorders and cancer [5]. These studies showed that some of the *Salvia* species containing terpenes, phenolics and flavonoids are the resources of homeopathic products [6].

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Therefore, most of the species are cultivated because of their medicinal properties [7]. Five *Salvia* species were studied here that are *Salvia suffruticosa*, *S. hydrangea*, *S. kronenburgii*, *Salvia xanthocheila* and *Salvia trichoclada*. The local names of those species are called Çeqlet, Mercanok, Çevrek, Guhbel, and Sevik, respectively [8].

Trace elements play a significant role in the formation of chemical constituents in plants [9]. It is known that twenty-three elements have physiological activities in mammals [10]. Some metals, such as, zinc, iron, copper, chromium and cobalt, are necessary at certain levels and they are toxic in high concentrations. On the other hand, some other metals; namely, mercury, lead and cadmium, are toxic even at low concentrations and have been known no useful properties. Determining of metal ions compositions of plants support their medicinal, nutrient and/or toxic properties [11, 12].

Trace elements have important roles in plant metabolism and biosynthesis as cofactors for the enzymes [13]. Medicinal plants are widely used in the treatment of human diseases and pain relief, due to their low adverse effects. Some medicinal plants and their mixtures may pose health risks owing to toxic elements contain. The contamination may become from environmental pollution [14]. For example, high levels of arsenic can result from the use of pesticides and fertilizers [15]. Human beings need metallic and non-metallic elements, within the permitted limits, for growth and health. Plants are an important medium for trace elements to transit from the soil to human beings [16]. Accordingly, the quality controls of these medicinal plants are important in terms of trace element content.

Graphite furnace atomic absorption spectrometry (GF-AAS) [17], flame atomic absorption spectrometry (F-AAS) [13,18-21], inductively coupled plasma optical emission spectrometry (ICP-OES) [22-24], inductively coupled plasma–mass spectrometry ICP-MS [25-27], techniques are used to determine the trace element contents of medicinal plants. When compared the mentioned techniques, ICP-MS, is a more effective technique in the determination of multiple elements at trace levels due to its high sensitivity, precision and large linear dynamic range.

The most common used chemometric techniques are Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA). PCA seeks for an answer about the relationship between the samples and the interaction between variables. However, Clustering technique (CA) provides information regarding the classification (characterization) of samples. These techniques reveal relationships of classification and the predictions that cannot be considered as "ordinary results"[28, 29].

This paper aims to determine toxic and nutrient elements concentrations of seeds, roots, leaves, flowers, and mixed samples of five *Salvia* species, collected from Van and Kars using ICP-MS. In addition, classification of trace metal components and evaluation of differences between sections were made by using PCA and HCA methods.

2. RESULTS AND DISCUSSION

2.1. Concentrations of elements in *Salvia* species

Seeds, roots, leaves, flowers, and mixed parts of five *Salvia* species were examined in this study for their metal contents, and the results were presented in Table 1.

The Lithium content was detected in the range of 0.28–11.33 mg kg^{-1}. The Li concentrations in the root samples of SH and also the Li concentrationsin the leaves samples of ST, SX, SK and SF's were higher than other samples. The richest Li content was detected in SX.

The Beryllium and vanadium contents were determined in the range of 2.01–122.16 µg kg^{-1} and 0.15–4.98 mg kg^{-1}, respectively. When compared to other parts, higher Be and V were observed in the roots of SF, SH, SX and ST and leaf of the SK. Among the *Salvia* species, SX contained the highest Be and V concentration than those of other *Salvia* species examined.

The Chromium content was detected to be in the range of 0.53–10.09 mg kg^{-1}. When compared to other parts, Cr was observed higher in the roots sections of SF, SH, ST the leaves samples of SK and SX's. Cr contents of the species SX and SF seem to be more than those of other *Salvia* species examined. The daily intake of chromium varies between 5 and 200 mg kg^{-1} [30]. In Tokalioglu's study, the Cr contents in 30 different medicinal herbs samples, consumed around Kayseri city was found to be in the range of 0.44 mg kg^{-1} (opium poppy) and 8.71 mg kg^{-1} (nettle) [11]. Abou-Arab and Donia [18] that taken 20 different types of spices and medicinal plant samples from Egypt was founded the Cr levels in the range of 2.47-33.75 mg kg^{-1}. Also, at the same study Abou-Arab and Donia that taken nine types of medicinal herbs from India was detected the Cr levels in the range of 0.96-8.19 mg kg^{-1} [18]. The limit values for chromium in Canada are determined to be 2 mg kg^{-1} and daily intake as 0.02 mg/day [31]. The Cr values in mixed samples of five different *Salvia* species

examined in this study, SFM, SHM, SKM, SXM and STM, were found to be 0.76, 2.16, 4.53, 4.65 and, 3.53 µg g^{-1}, respectively. The Cr potential in sage (*S. officinalis*) in Tokalioglu's [11] study was illustrated as 4.70.

When compared to other parts, more Co was observed in the roots sections of SF, SH and ST, flower section of SX and leaf section of SK, among species examined in this study. Co content of SX seems to be more than those of other *Salvia* species examined. It is also observed that Ni contents, however, were found to be more in the roots sections of SH and ST, flower section of SX, and leaves sections of SK and SF, when compared to other sections. It can be phrased that Ni content of SX and SK is more than those of other *Salvia* species examined. Co and Ni values for five different parts of these *Salvia* species were calculated to be in the range of 0.06-0.96 and 0.86-9.57 mg kg^{-1} while those values for mixed samples of five different *Salvia* species [SFM, SHM, SKM, SXM and STM] were found to be 0.96, 0.21, 0.42, 0.59, 0.43 mg kg^{-1} and 4.57, 1.99, 8.16, 4.05 and 2.70 mg kg^{-1}, respectively. In her study on 30 different medicinal herbs samples, consumed around Kayseri, Turkey, Tokalioglu [11] has reported the Co and Ni values to be in the range of 0.05-2.35 mg kg^{-1} and 0.72-13.1 mg kg^{-1}, respectively. Those values in sage (*S. officinalis*) sample, however, are reported to be 0.53, 3.83 mg kg^{-1}. The Co and Ni values in sage sample in Basgel and Erdemoglu [14] were found to be 0.34, 2.90 mg kg^{-1}. When compared to other sections, more Cu was determined in the leaf sections of SF and SK, root sections of SH and ST and stem section of SX among species examined in this present study. Cu content of SX seems to be the lowest of all, but all other *Salvia* species have close values.

Table 1. Quantification of studied trace elements in *Salvia* species by ICP-MS.

Concentrations of the analyzed metal elements (mean ± SD. n=3)										
SN*	**Li** (mg kg^{-1})	**Be** (µg kg^{-1})	**V** (mg kg^{-1})	**Cr** (mg kg^{-1})	**Co** (mg kg^{-1})	**Ni** (mg kg^{-1})	**Cu** (mg kg^{-1})	**Zn** (mg kg^{-1})	**As** (mg kg^{-1})	**Se** (mg kg^{-1})
SFS	0.39±0.03	2.34±0.11	0.17±0.01	0.68±0.05	0.06±0.00	1.00±0.08	5.51±0.17	9.13±0.34	0.05±0.00	0.61±0.01
SFR	4.39±0.23	53.84±2.25	4.98±0.33	10.09±0.72	1.08±0.07	1.22±0.09	10.97±0.21	2.29±0.16	0.41±0.02	1.05±0.02
SFL	11.40±0.51	10.50±0.85	1.08±0.05	4.08±0.23	0.38±0.02	5.14±0.12	24.74±0.34	5.92±0.28	0.25±0.01	1.17±0.02
SFF	1.04±0.03	15.24±0.48	0.92±0.03	3.59±0.21	0.39±0.03	5.07±0.17	11.00±0.22	2.85±0.18	0.12±0.01	1.04±0.02
SFM	7.47±0.42	33.14±1.24	2.81±0.24	0.76±0.05	0.96±0.06	4.57±0.24	8.14±0.19	3.53±0.11	0.38±0.02	0.74±0.01
SHS	0.75±0.04	2.01±0.01	0.15±0.01	0.53±0.03	0.04±0.00	0.86±0.05	8.89±0.17	6.84±0.24	0.05±0.00	1.20±0.02
SHR	3.01±0.21	79.72±3.21	4.75±0.23	7.09±0.42	0.94±0.02	6.01±0.23	20.53±0.36	0.52±0.01	0.50±0.02	0.89±0.01
SHL	1.73±0.32	8.59±0.47	0.51±0.04	0.96±0.05	0.10±0.01	1.12±0.13	8.27±0.18	4.56±0.19	0.13±0.01	1.58±0.02
SHF	0.59±0.04	7.53±0.06	0.47±0.03	0.99±0.05	0.12±0.01	1.58±0.16	8.68±0.19	5.14±0.24	0.09±0.00	1.25±0.02
SHM	0.90±0.06	25.87±1.54	1.10±0.11	2.16±0.18	0.21±0.02	1.99±0.16	7.83±0.17	2.63±0.23	0.14±0.01	1.13±0.02
SKS	0.28±0.02	3.25±0.02	0.28±0.01	0.91±0.05	0.11±0.01	1.34±0.10	7.48±0.17	4.00±0.20	0.07±0.00	0.82±0.02
SKR	0.38±0.03	6.38±0.47	0.56±0.03	1.68±0.15	0.26±0.01	2.67±0.12	9.24±0.19	2.55±0.16	0.08±0.00	0.84±0.02
SKL	1.64±0.11	31.29±1.98	1.75±0.19	6.74±0.39	0.68±0.05	9.06±0.39	11.05±0.23	1.54±0.11	0.38±0.03	1.05±0.02
SKF	1.03±0.08	20.17±1.18	1.35±0.14	5.38±0.37	0.59±0.04	8.22±0.34	9.62±0.20	1.96±0.14	0.19±0.01	0.95±0.01
SKM	1.09±0.21	17.30±1.58	1.25±0.23	4.53±0.38	0.42±0.05	8.16±0.27	7.92±0.17	1.25±0.18	0.35±0.02	1.00±0.02
SXS	3.68±0.17	19.98±1.74	1.27±0.09	2.90±0.17	0.41±0.03	2.85±0.12	9.91±0.17	3.55±0.20	0.15±0.01	0.87±0.01
SXR	6.16±0.28	122.16±4.56	3.19±0.28	3.73±0.27	0.73±0.05	3.25±0.19	3.42±0.07	0.76±0.05	0.61±0.04	0.48±0.01
SXL	11.33±0.52	18.02±1.62	3.09±0.29	5.10±0.28	0.71±0.05	5.03±0.15	4.42±0.8	0.74±0.03	0.72±0.04	0.53±0.01
SXF	7.70±0.34	30.91±1.21	2.57±0.26	7.27±0.30	0.80±0.04	9.75±0.26	6.31±0.16	1.83±0.18	0.66±0.04	1.00±0.02
SXM	9.34±0.32	46.96±2.25	3.02±0.25	4.65±0.34	0.59±0.02	4.05±0.24	2.94±0.06	0.96±0.06	0.62±0.04	0.51±0.01
STS	3.79±0.09	17.14±0.58	1.03±0.05	2.27±0.17	0.21±0.01	1.98±0.08	7.16±0.16	2.72±0.17	0.23±0.01	0.91±0.02
STR	2.24±0.01	72.39±2.38	2.77±0.11	4.13±0.33	0.47±0.02	3.49±0.15	17.61±0.27	1.41±0.12	0.56±0.02	0.90±0.02
STL	8.27±0.22	12.78±1.25	0.88±0.03	1.60±0.14	0.17±0.01	1.90±0.08	10.26±0.19	6.83±0.26	0.47±0.02	1.32±0.02
STF	1.44±0.02	60.85±3.52	2.15±0.14	3.76±0.25	0.39±0.01	3.27±0.19	9.26±0.15	2.53±0.19	0.26±0.01	1.10±0.02
STM	5.53±0.05	63.75±3.24	2.48±0.10	3.53±0.37	0.43±0.02	2.70±0.16	7.38±0.15	1.94±0.13	0.41±0.03	1.11±0.02

Concentrations of the analyzed metal elements (mean ± SD n=3)

SN*	Rb (mg kg^{-1})	Sr (mg kg^{-1})	Ag (µg kg^{-1})	Cd (µg kg^{-1})	Cs (µg kg^{-1})	Ba (mg kg^{-1})	Hg (mg kg^{-1})	Tl (µg kg^{-1})	Pb (mg kg^{-1})	U (µg kg-1)
SFS	2.41±0.12	60.52±3.20	20.17±1.00	4.40±0.25	2.98±1.45	50.20±2.24	0.22±0.01	1.62±0.04	0.11±0.01	2.10±0.10
SFR	3.30±0.15	311.43±9.25	44.29±2.50	34.05±1.55	94.40±4.52	153.29±6.48	0.50±0.01	15.92±0.46	0.61±0.03	58.53±2.50
SFL	3.75±0.15	20.24±1.50	59.64±3.50	21.30±1.00	32.45±1.68	8.79±0.42	1.51±0.05	7.80±0.28	2.94±0.13	13.63±1.00
SFF	9.02±0.50	184.29±5.50	119.05±6.50	10.31±0.50	29.17±1.15	9.90±0.46	1.29±0.05	3.42±0.18	0.30±0.01	9.55±0.50
SFM	5.50±0.25	144.73±7.25	37.62±2.50	30.61±1.85	74.53±4.52	108.46±4.24	0.44±0.01	15.32±0.41	0.97±0.04	33.70±1.16
SHS	9.10±0.50	202.73±10.50	38.00±2.50	14.55±0.65	21.10±1.13	49.00±2.44	0.24±0.01	28.31±0.24	0.40±0.01	2.11±0.10
SHR	9.95±0.50	215.32±10.50	49.70±2.50	64.80±3.34	122.44±5.89	78.58±3.45	0.86±0.03	35.14±0.26	5.21±0.26	70.01±3.25
SHL	4.58±0.20	660.27±12.25	32.25±2.50	15.03±0.70	21.03±1.13	79.75±3.63	0.47±0.01	22.76±0.19	0.35±0.01	7.82±0.35
SHF	19.71±0.10	223.94±5.25	34.40±2.50	11.60±0.50	35.60±1.68	28.93±1.43	0.40±0.01	15.41±0.44	0.38±0.01	5.04±0.30
SHM	7.42±0.30	193.84±10.50	38.90±3.00	28.42±1.42	39.37±1.87	40.15±2.00	0.64±0.03	25.12±0.13	0.62±0.03	15.28±0.78
SKS	2.22±0.10	176.06±10.12	16.00±1.00	24.61±1.27	6.61±0.28	55.45±2.63	0.56±0.01	1.70±0.14	0.22±0.01	4.35±0.20
SKR	3.48±0.05	179.67±10.25	28.90±1.50	32.55±1.54	12.35±0.56	55.11±2.50	1.56±0.05	2.71±0.18	0.38±0.01	7.23±0.40
SKL	5.00±0.20	454.38±13.50	57.64±2.50	22.15±1.17	64.22±3.15	92.23±3.78	1.04±0.04	8.06±0.21	0.60±0.03	25.72±1.25
SKF	10.47±0.05	142.56±7.50	98.40±5.00	13.00±0.56	40.95±2.05	20.25±1.00	1.13±0.04	3.67±0.14	0.68±0.01	14.02±0.60
SKM	6.67±0.05	410.56±15.25	66.67±3.50	22.80±1.16	47.90±3.27	72.10±4.85	0.69±0.02	7.95±0.28	0.50±0.05	25.29±1.50
SXS	9.71±0.50	48.22±2.25	36.00±1.80	22.70±1.17	63.55±3.27	90.22±3.85	0.35±0.01	7.31±0.34	1.40±0.10	10.22±0.65
SXR	7.66±0.35	65.61±3.50	31.79±1.50	62.00±3.21	580.40±16.79	181.53±7.25	0.15±0.01	45.39±0.28	1.97±0.19	105.60±4.85
SXL	9.91±0.50	105.85±4.50	56.50±2.50	46.10±2.36	420.36±8.49	112.80±4.90	0.37±0.01	15.60±0.65	1.05±0.09	36.53±1.85
SXF	28.21±0.15	28.59±2.50	102.54±5.25	24.60±1.28	134.09±6.42	27.67±1.28	0.44±0.01	3.95±0.25	0.21±0.01	8.80±0.50
SXM	9.72±0.50	65.31±3.50	49.78±2.50	55.92±3.47	522.55±14.65	125.82±5.50	0.26±0.01	34.90±0.26	1.50±0.05	71.80±3.45
STS	4.99±0.25	30.48±1.50	63.63±3.00	21.60±1.10	47.27±2.45	27.20±1.35	0.20±0.01	8.41±0.47	0.62±0.04	33.10±1.85
STR	3.63±0.05	56.77±2.50	58.22±3.00	51.84±2.58	135.94±6.75	44.00±2.12	1.56±0.06	26.75±0.20	4.92±0.02	133.90±5.25
STL	3.55±0.15	52.65±2.25	27.88±1.50	25.42±1.26	42.10±2.01	25.64±1.15	0.61±0.02	6.22±0.36	0.51±0.03	22.91±1.25
STF	8.50±0.20	13.98±0.65	120.23±6.50	42.43±2.24	128.45±5.56	93.01±3.75	0.81±0.03	15.00±0.15	1.09±0.12	54.83±2.75
STM	4.19±0.20	46.01±2.50	62.93±3.00	49.72±2.46	101.86±7.24	28.93±1.35	0.22±0.01	20.55±0.13	1.27±0.02	69.81±3.55

*: Sample Name

When compared to other parts, however, more Zn content was observed to be in the seeds sections of SF, SH, SK and SX and leaf part of ST, among these species examined in this study. It is apparent that Zn contents of SF and SH samples are more than those of other *Salvia* species. Cu and Zn values for five different parts of these *Salvia* species were calculated to be in the range of 2.94-24.74 and 0.52-9.13 mg kg^{-1} while those values for mixed samples of five different *Salvia* species [SFM, SHM, SKM, SXM and STM] were found to be 8.14, 7.83, 7.92, 2.94 and 7.38 mg kg^{-1} and 3.53, 2.63, 1.25, 0.96, 1.94 mg kg^{-1}, respectively. In her study on 30 different medicinal herbs samples, consumed around Kayseri, Turkey, Tokalioglu [11] has reported the Cu and Zn contents to be in the range of 3.32-30.2 mg kg^{-1} and 3.75-88-13.1 mg kg^{-1}, respectively. Those values in sage (*S. officinalis*) sample, however, are reported to be 6.66 mg kg-1 and 35.8 mg kg^{-1}. The Cu and Zn contents in Basgel and Erdemoglu's[14] research on seven different medicinal plants consumed in Turkey are reported as 3.92-35.8 mg kg-1 and 21.9-48.4 mg kg^{-1}. Values in sage sample in Basgel and Erdemoglu[14] additionally, were found to be 35.8 and 48.4 mg kg^{-1}. In another research, Maiga, et. al.[13] was report the Cu and Zn values in seven medicinal plants as 2.4-17.1 mg kg^{-1} and 9.2-67.1 mg kg^{-1}, respectively.

The Arsenic content was determined to be in the range of 0.05-0.72 mg kg^{-1}. When compared to other parts, however, the higher As content was observed to be in the root sections of ST, SF and SH and leaf sections

of SK and SX, among species examined in this study. The As value of SX seems to be higher than those of other *Salvia* species.

The Selenium content was determined to be in the range of 0.48-1.58 mg kg^{-1}. In comparison to other parts, however, more Se content was observed to be in the leaf sections of ST, SF, SK and SH and flower section of SX. Se content of SX seems to be the lowest of all, but all other *Salvia* species have close Se values.

More Rb, when compared to other sections, was determined in flower sections of all *Salvia* species examined in this study. The content of Rb for SX appears to be higher than that of other *Salvia* species. However, Sr contents were determined to be higher in the flower sections of SX, SH and SK, and root sections of ST and SF, in comparison to other sections. In terms of Sr contents, SH has the highest concentration. Sr and Rb values for five different parts of these *Salvia* species were calculated to be in the range of 20.24-660.27 mg kg^{-1} and 2.22-28.21 mg kg^{-1} while those values for mixed samples of five different *Salvia* species [SFM, SHM, SKM, SXM and STM] were found to be 144.73, 193.84, 410.56, 65.31, 46.1 mg kg^{-1} and 5.50, 7.42, 6.67, 9.72, 4.19 mg kg^{-1}, respectively. The Sr contents in the study of Basgel and Erdemoglu's [15] research on seven different medicinal plants consumed in Turkey are reported 16.5- 69.2 kg^{-1}. Values in sage sample in Basgel and Erdemoglu[14], additionally, were found to be 17.5 mg kg^{-1}. The Sr and Rb contents in sage (*S. officinalis*) sample, in Tokalioglu's [11] study on 30 different medicinal herbs samples, consumed around Kayseri, Turkey, were reported to be 46.2 mg kg-1 and 3.67 mg kg^{-1}, respectively.

The Silver content was determined to be in the range of 16.00-120.23 µg kg-1. When compared to other parts, more Ag content was observed to be in the flower sections of SF, SK, SX and ST and the root section of SH. The Ag value of SF and ST seems to be higher than those of other *Salvia* species.

The Cadmium content was determined to be in the range of 4.40-64.80 µg kg^{-1}. When compared to other parts, more Cd content was observed to be in the root sections of all *Salvia* species. The Cd content of SX and ST appeared to be higher than those of other *Salvia* species.

The Cesium, tellurium and uranium contents were determined to be in the range of 2.98-580.40 µg kg^{-1}, 1.62 - 45.39 µg kg^{-1} and 2.10-133.90 µg kg^{-1}. When compared to other parts, more Cs, Tl and U content were observed to be in the root sections of SF, SH, SX and ST and the leaf section of SK. It is observed that Cs content in SX, Tl in SH and U in ST is higher than those of other *Salvia* species.

The Barium content was determined to be in the range of 8.79-153.29 mg kg^{-1}. When compared to other parts, more Ba content was observed to be in the root sections of SF, SH and SX, leaf section of SK and flower section of ST. The Ba content of SX appeared to be higher than that of other *Salvia* species.

The Mercury content was determined to be in the range of 0.15 - 1.56 mg kg^{-1}. When compared to other parts, more Hg content was observed to be in the root sections of ST, SK and SH, leaf section of SF and flower section of SX. The Hg content of SK appeared to be higher than that of other *Salvia* species.

The Lead content was determined to be in the range of 0.11-5.21 mg kg^{-1}. When compared to other parts, more Ba content was observed to be in the root sections of ST, SX and SH, leaf section of SF and flower section of SK. The Pb content of ST and SH appeared to be higher than that of other *Salvia* species. Pb values for mixed samples of five different *Salvia* species we examined in this study [SFM, SHM, SKM, SXM and STM] were found to be 0.97, 0.62, 0.50, 1.50 and 1.27 mg kg^{-1}, respectively. The Pb value in sage sample in the study of Basgel and Erdemoglu [14] was reported to be 1.14 µg g^{-1} and the Pb value in sage (*S. officinalis*) sample, in Tokalioglu's[11] study was found to be 1.44 mg kg^{-1}.

Countries in different regions of the world have determined different limits for toxic metals on medicinal plants. For samples of raw herbal materials, the toxicity limits for lead, arsenic, chromium and cadmium are reported to be 10, 5, 2 and 0.3 ppm, respectively. As for the finished herbal products, the toxicity limits are designated as 0.02 mg/day for lead, chromium and mercury, 0.01 mg/day for arsenic, and 0.006 mg/day for cadmium [31]. The values for arsenic, lead, cadmium and mercury in all *Salvia* species examined are lower than the limit values designated for raw herbal materials. The chromium content, however, is higher than 2 mg kg^{-1}, which is the limit value for raw herbal materials. Therefore, it is important to control the dosage of these species in terms of chromium.

The toxicity of metals depends on the oxidation state and concentration. As, Cd, Cr, Hg and Pb metals are toxic metals for human beings. While the fact that these toxic metals are generally accumulated at the root sections of *Salvia* species is an advantage for consumers, it appears that the dosage control of *Salvia* species in medical use is of paramount importance since the cadmium and lead content in the leaf sections of SF, the

mercury content in the flower section of SX, the lead content in the flower section of SK are high in comparison to other sections, and these toxic metals exist in every part of *Salvia* species.

2.2. Principal Component Analysis (PCA)

The purpose of the chemometric analysis is to make estimates by reducing the number of dimensions (data) and summarize the data in tables and graphs. In this study, twenty metal contents of five different *Salvia* species were analyzed. Big data is almost difficult to interpret. For this reason, to decrease the number of data and to turn big data into visual graphics make the interpretation easier. While visualizing all information in the PCA dataset via graphics, the differences between the samples could be detected. At the same time, the graph provides an evaluation of the main components that affect the samples.

PCA results are summarized in Table 2. As a result of principal component analysis performed by twenty heavy metals, 6 components having eigenvalues greater than 1 were obtained. According to the PCA results of *Salvia* species, the first six principal components explain 89.60% of the total variance. The first principal component 1 (PC1), second principal component 2 (PC2), third principal component 3 (PC3) and fourth principal component 4 account for 40.3%, 15.0%, 12.8 % and 9.80% of total variance, respectively. The percentage for other principal components gradually decreases. Those values which were shown in bold in Table 5 were more effective than others in explaining the principal components. The first principal component indicates the highest variance in the data set. The dominant variables were Be, V, Co, As, Cd and U for PC1, Cs and Tl for PC2, Cu, Hg and Pb for PC3, Ag for PC4 and Li and Zn for PC5.

Table 2. The loading, eigenvalue, variance and cumulative variance data of the principal components analyses (PCA).

Metal	PC1	PC2	PC3	PC4	PC5	PC6
Li	0.1761	0.0474	-0.1245	0.0328	**0.7005**	-0.2197
Be	**0.2993**	0.0614	0.1481	0.0632	-0.2670	-0.0703
V	**0.3252**	-0.1129	0.0063	-0.0608	-0.0013	-0.0672
Cr	0.2499	-0.3597	-0.1413	-0.1287	0.0250	0.0046
Co	**0.2892**	-0.2627	-0.1423	-0.1367	0.0251	0.0435
Ni	0.1753	-0.4259	-0.2183	-0.1655	0.0257	0.0382
Cu	0.0002	-0.3074	**0.4636**	0.0106	0.3006	-0.0853
Zn	-0.2766	0.0872	0.1080	-0.0507	**0.3144**	-0.3173
As	**0.3131**	-0.0019	-0.0866	0.1546	0.0810	-0.1677
Se	-0.1908	-0.2078	0.1643	-0.0596	-0.1218	-0.5930
Rb	0.0113	-0.0737	-0.3542	0.3101	-0.0569	-0.4304
Sr	-0.0558	-0.1595	0.0524	-0.5557	-0.2726	-0.2544
Ag	0.0495	-0.3238	-0.1148	**0.4644**	-0.2025	-0.0902
Cd	**0.3019**	0.1646	0.1638	0.0820	-0.0183	-0.0104
Cs	0.2729	**0.2824**	-0.1288	0.0565	0.0562	-0.0465
Ba	0.2182	0.1206	-0.0680	-0.4756	0.1272	0.0487
Hg	-0.0382	-0.3362	**0.3387**	0.1342	0.0287	0.2560
Tl	0.2115	**0.2691**	0.2055	-0.0682	-0.2302	-0.3496
Pb	0.1991	-0.0484	**0.4529**	0.0924	0.1209	-0.0259
U	**0.2849**	0.1067	0.2492	0.1027	-0.1371	0.0072
Eigenvalue	8.06	3.00	2.57	1.95	1.18	1.15
Variance (%)	40.30	15.00	12.80	9.80	5.90	5.70
Cumulative (%)	40.30	55.30	68.20	77.90	83.80	89.60

Table 3 presents the scores of the first sixth rotated principal components for each *Salvia* sample. When the scores were analysed, it was observed that Be, V, Co, As, Cd and U concentrations were higher in SXR, SHR, SXM, SFR, SXL, STL and SFM while all were lower in SHS, SFS, SHF, SHL, SKS, STS and SFF samples. Similarly, It can be said that Cs and Tl concentrations for the second principal component in SXR and SXM samples are higher when compared to those in other samples. Cu, Hg and Pb concentrations for the PC3 are the highest in STL, SHR and SFL samples, Ag concentration for the PC4 is the highest in STF and SXF samples. The values, which were shown in bold in Table 3 were more effective than others in explaining the score values of the principal components.

Table 3. The score values of the principle components analyses (PCA).

Samples	PC1	PC2	PC3	PC4	PC5	PC6
SFS	-3.4712	1.9264	-0.7182	-0.3482	0.2001	1.9576
SFR	**3.2143**	-2.2246	-0.7321	-2.7988	-0.0205	-0.1377
SFL	-1.1373	-1.9946	**2.5365**	-0.1503	**3.4341**	-0.6612
SFF	-2.1489	-1.9508	-0.3447	2.0710	-0.5420	0.8017
SFM	**2.1023**	-0.8462	-1.3483	-1.3587	0.8893	0.7208
SHS	-3.6261	1.7021	0.3626	-0.4136	-0.2579	-1.4940
SHR	**4.4644**	-1.2280	**3.2101**	-0.2680	-0.3274	-0.3189
SHL	-3.2320	0.6651	0.8170	-2.5667	-1.1988	-2.1307
SHF	-3.4068	0.7904	-0.5582	0.3176	-0.5564	-1.7150
SHM	-1.6756	0.3900	0.6693	0.3336	-1.1917	-0.1402
SKS	-3.1122	1.4001	0.0915	-0.7443	0.0434	1.5011
SKR	-2.3663	-0.0117	0.7780	-0.2618	-0.1559	2.0457
SKL	0.2483	-2.2055	-0.2941	-1.7302	-0.7851	0.3759
SKF	-1.0349	-2.3969	-0.8866	1.0372	-0.7454	0.8123
SKM	0.8113	-2.2015	-1.1052	-1.8855	-1.0238	0.2269
SXS	-1.1772	0.7053	-0.3983	-0.1371	0.7757	0.4070
SXR	**5.7735**	**3.8171**	-0.0471	-0.4891	-0.7920	-0.0711
SXL	**3.1772**	1.1953	-2.1539	0.1973	1.5494	0.3579
SXF	1.2581	-2.2017	-3.6873	**2.3064**	0.4109	-1.6062
SXM	**4.2915**	**2.7682**	-1.1328	0.3060	0.4905	-0.0523
STS	-2.5669	0.8957	0.6258	0.2701	1.9470	-0.9534
STR	-1.5429	0.9816	-0.4694	0.9531	0.0052	0.5667
STL	**2.9598**	-0.3487	**4.1409**	1.6438	-0.4327	0.5171
STF	0.5075	-0.4985	0.3856	**2.6203**	-1.3025	-0.3483
STM	1.6899	0.8714	0.2587	1.0958	-0.4136	-0.6617

In Figure 1 the score plot graphic was given for *Salvia* species. SFS, SKS, SHS, STS and SXS samples were significantly separated from the other samples. SHL, SHM and SHF parts of *Salvia* species, and SKR and STR parts were similar to the stem sections of the other samples, and SFS, SKS, SHS, STS, SXS, SHL, SHM, SHF, SKR and STR samples belong to the same group. In addition, it was also observed that SXR, SXL, SXM and STM set another group. It can also be said that SHS, SHL, SHM and SHF samples of *Salvia* hydrangea collected from Kars, form another group. SFF, SFL, SKF, SKL, SKM, SXF and STF samples constitute another group, as well. As seen in Figure 1. the flower sections of all *Salvia* species except SHF, were also in a distinct group. SFM, SFR, SHR and STL were also samples belonging to another group.

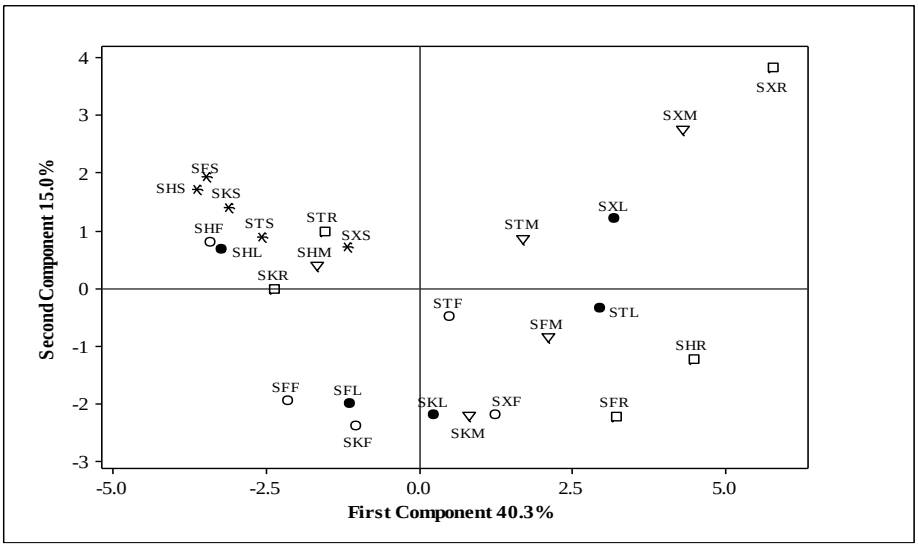

Figure 1. Score plot graphic for principal component analysis (PC1 and PC2) in *Salvia* species, ∗stem, □ root, . • leaf, o flower and ∇ mixed.

When Figure 1 and Figure 2 were interpreted together, it was observed that Se and Sr scores of SXR, SXL and SXM samples were low while Tl and Cs scores high. It appears that SXR, SXL and SXM samples provided high correlation with PC2. The stem sections of the samples, however, were not characterized by any heavy metals. SHL, SHF, and SHM samples were characterized by Zn. It was found that, and samples were characterized by and

It was found that SFR, SFF, SFM, SKL, SKF, SKM, SHR, STL and STF samples were characterized by Ni, Sr, Cr, Cu, Hg, Ag, Co, V and Rb respectively.

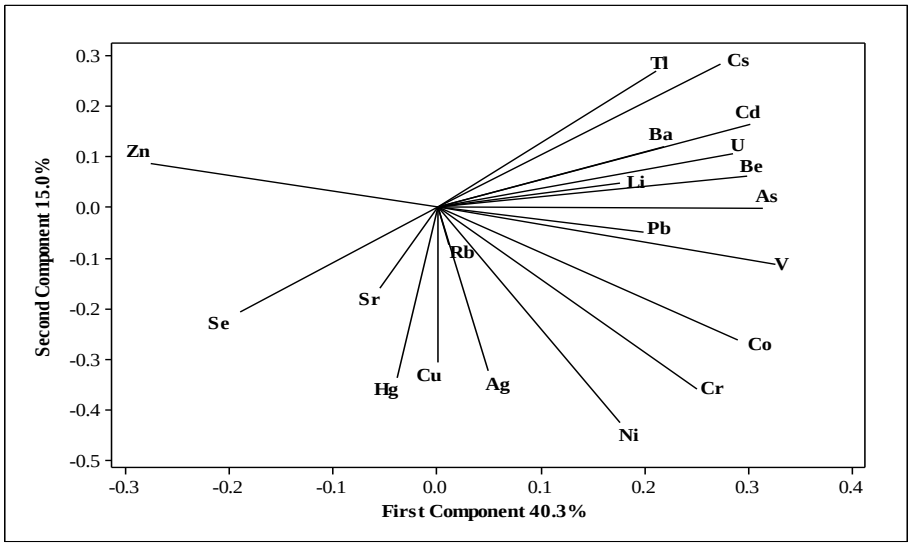

Figure 2. Loading plot for principal component analysis (PC1 and PC2) in *Salvia* species.

Figure 1 displays the results of cluster analysis (HCA) performed to determine the degree of closeness of *Salvia* species divided into five different sections. Cluster analysis (HCA) was performed in order to compare the samples taken from two different regions, the distribution of heavy metals in five different sections of these samples and to make comparisons regarding the PCA results. The measurement is based on the Euclidean distance. In this study, The Ward method was used as a clustering method. Dendrogram obtained from the Ward linkage method is shown in Figure 3.

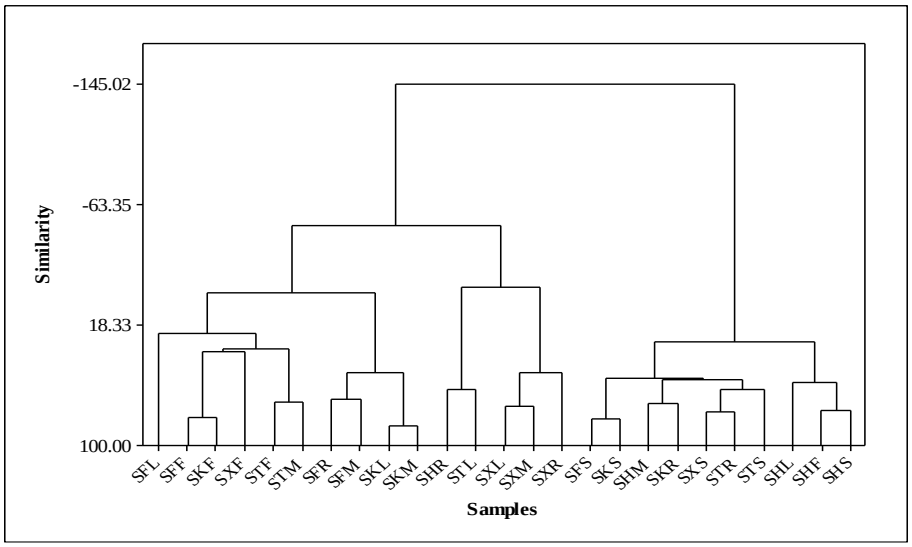

Figure 3. Dendrograms obtained by Euclidean distance and Ward Linkage methods.

When Figure 3 was examined, it was observed that similar results to those of PCA were obtained and five clusters were formed. The first cluster consists of 6 samples; SFF, SKF, SXF and STF samples (flowers) and SFL and STM are included in this group. The second cluster includes 10 samples; these were SFS, SHS, SKS,

SXS and STS (seeds) samples and SHL, SHM and SHF samples (*S. hydrangea*) and STR and SKR samples. The third one includes 4 samples, namely SFR, SFM, SKL and SKM samples. Two samples, SHR and STL, are in the fourth cluster. The last cluster was comprised of 3 samples; SXR, SXL and SXM. Cluster 2 samples seemed to be exactly the same as the PCA results.

3. CONCLUSION

In this study, twenty (Li, Be, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Hg, Tl, Pb, and U) trace elements contents of five S. species, collected from the Van and Kars cities in Turkey, were investigated for the first time. Since S. species are consumed as herbal tea and medicinal plants by the people, it is important to determine the trace element content from the point of consumers. ICP-MS technique, used in trace element analysis after microwave digestion, is a fast, accurate, precise and sensitive method.

The toxicity of metals depends on the oxidation state and concentration. As, Cd, Cr, Hg and Pb metals are toxic metals for human beings. In our study, the values for lead, arsenic, and cadmium in all *Salvia* species examined are lower than the toxicity limit values designated for raw herbal materials (10, 5 and 0,3 ppm, respectively). Our results show that, toxic metals are generally accumulated in the plants' root sections, while the fact that these toxic metals are generally accumulated at the root sections of *Salvia* species is an advantage for consumers, it appears that the dosage control of *Salvia* species in medical use is of paramount. Also the cadmium and lead content in the leaf sections of SF, the mercury content in the flower section of SX, the lead content in the flower section of SK are high in comparison to other sections. When the stem, leaves, flower, root and mixed sections of Salvia species examined in this study were analyzed, as seen that the content of chromium in some parts is higher than 2 mg kg^{-1}, which is the limit value for raw herbal materials. Therefore, it is important to control the dosage of these species in terms of chromium.

And all the studied Salvia species are illustrated to be resources to meet the amount of nutritive element requirements to be taken on a daily basis (the daily requirements of a person weighing 70 kg have been detected for Zn (15 mg/day), Cu (2.5 mg/day), Sr (1.6 mg/day) and Ni (0.025 mg/day)).

Considering all the results, it is concluded that these Salvia species can be consumed as medicinal plants and herbal tea provided that dose control.

The first two components in PCA performed for *Salvia* species and sections were sufficient to explain 55.30% of the total variance. According to the PCA results, the root sections of SH, ST, SX, SK and SF samples emerged in a factor and SHL, SHM, SHF, SKR and STR samples were similar to this group. HCA also supported these findings. It was observed that the Zn content of species in this group was higher than other samples. Other heavy metal concentrations were found to be much lower. It can also be inferred that the flower sections of ST, SX, SK and SF samples were clustered in a group and the leaf section of SF sample appears to be similar to this cluster. The leaf, root and mixed sections of SX sample were also included in a single cluster. The mixed section of ST is similar to that cluster. Cs and Tl elements are heavy metals which affect this group most. Similar findings were obtained as results of PCA and HCA.

4. MATERIALS AND METHODS

4.1. The plant materials

The aerial parts and roots (whole Salvia species) of *S. suffruticosa, S. hydrangea, S. kronenburgii, S. xanthocheila* and *S. trichoclada* were collected from southeastern Turkey (Gurpınar, Van; Kagizman, Kars; Gurpınar, Van; Gurpınar, Van and Catak, Van; respectively) in July 2014, and identified by M. Firat. Voucher specimens were deposited in the Herbarium of Van Yuzuncu Yil University, Faculty of Education (Table 4.).

4.2. Instruments

An Agilent 7700X ICP-MS (Tokyo, Japan) was used to determine As, Ag, Ba, Be, Cd, Co, Cr, Cu, Cs, Hg, Li, Na, Pb, Rb, Se, Sr, Tl, U, V, and Zn in the samples. Digestion procedure of the studied samples prior to analysis was carried out in a Milestone Start D Brand microwave (Denmark) oven equipped with PTFE vessels.

4.3. Reagents and solutions

For analytical purity was used ultra-pure nitric acid (Merck, Darmstadt, Germany) and ultra-pure hydrogen peroxide (Merck, Darmstadt, Germany). Ultrapure deionized water (18,2 MΩ) was used in all experiments. In the ICP-MS measurements were used ^{45}Sc, ^{72}Ge, ^{115}In and ^{209}Bi at 200 µg L^{-1} concentration as mixed internal standards. After diluted at 10 mg L^{-1} mix standard in the concentration range of 0-100 µg $L^{-1,}$

the calibration curves were prepared for As, Ag, Ba, Be, Cd, Co, Cr, Cu, Cs, Hg, Li, Ni, Pb, Rb, Se, Sr, Tl, U, V, and Zn metals. Internal and calibration standards were obtained from Agilent Technologies (USA). The accuracy and precision of the method were evaluated by using a CRM NIST 1573a Tomato Leaves (National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) Certified Standard Reference Material.

Table 4. Information about of studied *Salvia* species.

Sample names	Sample abbreviations	Collection location	Collection time	Herbarium number
Salvia suffruticosa seed	SFS			
Salvia suffruticosa root	SFR			
Salvia suffruticosa leaf	SFL			
Salvia suffruticosa flower	SFF			
Salvia suffruticosa mixed	SFM	Gurpınar Van	July 2014	VANF-164079
Salvia hydrangea seed	SHS			
Salvia hydrangea root	SHR			
Salvia hydrangea leaf	SHL			
Salvia hydrangea flower	SHF			
Salvia hydrangea mixed	SHM	Kagizman Kars	July 2014	VANF-164080
Salvia kronenburgii seed	SKS			
Salvia kronenburgii root	SKR			
Salvia kronenburgii leaf	SKL			
Salvia kronenburgii flower	SKF			
Salvia kronenburgii mixed	SKM	Gurpınar Van	July 2014	VANF-164081
Salvia xanthocheila seed	SXS			
Salvia xanthocheila root	SXR			
Salvia xanthocheila leaf	SXL			
Salvia xanthocheila flower	SXF			
Salvia xanthocheila mixed	SXM	Gurpınar Van	July 2014	VANF-164082
Salvia trichoclada seed	STS			
Salvia trichoclada root	STR			
Salvia trichoclada leaf	STL			
Salvia trichoclada flower	STF			
Salvia trichoclada mixed	STM	Catak Van	July 2014	VANF-164083

4.4. Sample preparation

The studied *Salvia* species were divided into groups as seeda, roots, leaves, flowers and mixed, washed by tap water first and deionized water secondly, and dried at 70 ^{0}C' for 48 hours. Parts of the species were coded as *S. suffruticosa* (SF), *S. hydrangea* (SH), *S. kronenburgii* (SK), *S. xanthocheila* (SX) and *S. trichoclada* (ST). Additionally, the seed, root, leaf, flower and mixed groups were encoded as (S), (R), (L), (F) and (M) respectively. Then, the dried *Salvia* species were pulverized by a blender. About 200 mg of pulverized samples were accurately weighed into PTFE digestion vessels and 6 mL HNO_3 and 2 mL H_2O_2 were added; then, they were digested in a microwave oven. The digested samples were taken into 25 mL volumetric flasks and filled by deionized water. Blank tests were carried out as three independent experiments in the same way. The certified standard reference material CRM 1573a Tomato Leaves (National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) was applied for the same digestion method mentioned above.

4.5. Method validation

Linear range, regression correlation coefficient (R), LOD and LOQ values regarding the calibration curve drawn for twenty elements under optimized working conditions are presented in Table 5. The fact that r^2 value appears to be higher than 0. 99 indicate that linearity is acceptable. The limits of detection and quantification for the metals were calculated using standard deviation (σ_{blank}) of 10 independent blank solutions and the slope (m) of the calibration graph to obtain LOD or LOQ = αx (σ/m); where a is equal to 3 for the LOD and 10 for the LOQ (Table 5).

Table 5. Analytical parameters of the applied ICP-MS method used to quantify the trace elements in *Salvi* species (linear range. regression correlation coefficient (R). limit of detection (LOD) and limit of quantification (LOQ)).

Element	Linear range (μg kg^{-1})	Regression	Correlation coefficient (*r*)	Limit of detection (μg kg^{-1})	Limit of quantification (μg kg^{-1})
Ag	1-100	y= 0.4710 x - 2.2430	0.9890	0.2356	0.7068
As	1-100	y= 0.0783 x + 0.1780	0.9901	0.1070	0.3210
Ba	1-100	y= 0.0121 x + 0.0130	0.9993	0.2249	0.7489
Be	1-100	y= 0.0246 x + 0.1692	0.9909	0.0213	0.0639
Cd	1-100	y= 0.0334 x + 0.0290	0.9963	0.0132	0.0396
Co	1-100	y= 0.1219 x + 0.0901	0.9907	0.2202	0.6606
Cr	1-100	y= 0.1028 x + 0.3012	0.9989	0.2175	0.6525
Cs	1-100	y= 0.0480 x - 0.0168	0.9895	0.0310	0.0930
Cu	1-100	y= 0.0658 x + 0.1028	0.9999	0.0200	0.0600
Hg	1-100	y= 0.0111 x - 0.0124	0.9860	0.0543	0.1629
Li	1-100	y= 0.1057 x - 0.0442	0.9989	0.1216	0.3648
Ni	1-100	y= 0.0277 x + 0.0370	0.9999	0.3108	0.9324
Pb	1-100	y= 0.1860 x + 0.2304	0.9936	0.0832	0.2777
Rb	1-100	y= 0.1290 x + 0.1301	0.9891	0.2873	0.8634
Se	1-100	y= 0.0025 x + 0.0070	0.9940	0.2321	0.6963
Sr	1-100	y= 0.1912 x + 0.4047	0.9912	0.0896	0.2688
Tl	1-100	y= 0.1379 x - 0.1230	0.9991	0.2551	2.7653
U	1-100	y= 0.1530 x + 0.0646	0.9898	0.0044	0.0146
V	1-100	y= 0.1221 x + 0.0849	0.9992	0.2770	0.8310
Zn	1-100	y= 0.0717 x + 0.1806	0.9966	0.3085	0.9255

Findings regarding the certified standard reference material CRM NIST 1573a Tomato Leaves (National Institute of Standards and Technology, Gaithersburg, MD, USA) analyzed to evaluate the accuracy and precision of the method are presented in Table 6.

Table 6. Accuracy assessment of analysis of CRM NIST1573a Tomato Leaves.

Elements	Certified (mg/kg)	Found (mg/kg)	Recovery (%)
As	0.122±0.004	0.128±0.005	104
Ba	63±2	64±1	102
Cr	1.99±0.06	2.00±0.07	100
Cd	1.52±0.04	1.49±0.04	98
Co	0.57±0.02	0.56±0.06	98
Cu	4.70±0.14	4.76±0.08	101
Fe	368±7	375±6	102
Hg	0.034±0.004	0.032±0.003	94
Ni	1.59±0.07	1.57±0.11	99
Rb	14.89±0.27	15.19±	102
Se	0.054±0.003	0.051±0.003	94
U	(0.035)[a]	0.034±0.001	97
V	0.835±0.010	0.826±0.008	99
Zn	30.9±0.7	31.3±0.2	101

a:Values in parentheses are not certified values, Values expressed are means ± standard deviation of three parallel measurements ($p < 0.05$).

4.6. The chemometric analysis

The chemometric analyses of metal contents of seeds, roots, leaves, flowers and mixed parts of five *Salvia* species were carried out using principal component analysis (PCA) and hierarchical clustering analysis

(HCA), which are multivariate data analysis methods. Both methods for clustering and classification are mainly based upon the principal component analysis. PCA reduces multiple variables into a set of fewer components created by their linear combinations by hindering correlations between those examined variables. PCA-based methods can classify the samples by clustering into various groups. HCA classifies samples in a given data set and defines those data according to their similarities. HCA can be applied directly to the original variables, as well as possible to be applied to the results obtained from PCA, in case of existing too many variables. In this study, HCA applied to the results of the analysis of trace metal components, the measurement is based on the Euclidean distance. The Ward's method was used as a clustering method. In this context, all classification and clustering analyses for *Salvia* species were carried out using Minitab statistical software.

4.7. Statistical Method

All statistical calculations were made using Minitab 16.2.1 statistical software (Minitab Inc. 2010). In this study the sections (seeds, leaves, flowers, roots and mixed) of the five *Salvia* species were classified multivariate analysis regarding twenty trace metal components in *Salvia* species were carried out using PCA and HCA techniques.

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