

# Toxicology of the Various Elements in the Decoction Samples of Lemon Balm and Sage Species

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## Abstract

Plants are used for many purposes from past to present. One of them is consumed by people for therapeutic purposes. Some compounds or excessive heavy metal intake during consumption can be harmful to human health. In this study, the amount of Aluminum (Al), Nickel (Ni) and Cobalt (Co), which are toxic elements in the decoction samples obtained with different sample amount (g) and decoction time (min) of *Melissa officinalis* L. (lemon balm) and *Salvia officinalis* L. (sage) were investigated. All elements were analyzed by Inductive Coupled Plasma-Mass Spectrophotometer (ICP-MS) at Yozgat Bozok University Science and Technology Application and Research Center. The calibration curve was plotted with 11 points. In both species, the amount of Al was observed to be higher than the other elements. The highest amount of Al was observed in sage plant (1155.6 ppb), while the highest amount of Ni was found in lemon balm (771.3 ppb). In contrast, the maximum amount of Ni and Al was found in the decoction samples of the lemon balm. There was no statistically significant difference between the amounts of Co element in lemon balm and sage decoction samples. In general, increased amount and increased application time of lemon balm plant were found to cause an increase in Al and Ni amounts. As a result, toxicological evaluation of such plants used for their therapeutic properties is important for human and public health.

**Keywords:** Sage; Lemon balm; Toxicology; ICP-MS; Decoction.

## Introduction

Medicinal sage (*Salvia officinalis* L.) and lemon balm (*Melissa officinalis* L.) plants are among the most consumed herbal teas. Furthermore, they are medicinal and aromatic plants rich in rosmarinic acid, an important flavonoid compound in both species.<sup>10,8</sup> While the therapeutic and antioxidant components of bioactive compounds are rosmarinic

acid, caffeic acid and derivatives, chlorogenic acid, ferulic acid, gallic acid, quercetin, quercitrin and routine in lemon balm plant,<sup>5,11,12</sup> in medical sage, it has been found that compounds such as rosmarinic acid, ellagic acid, caffeic acid, routine, chlorogenic acid and quercetin are present.<sup>4,8</sup> Many scientists have reported that these compounds exhibit numerous biological activities.<sup>7,2,13,9</sup> Therefore, parts of these species such as herba, leaf are actively used in many fields such as traditional medicine, cosmetics, medicine, food and perfumery.<sup>1</sup> In particular, the use of herbal medicines to alleviate or treat human disease is increasing day by day in many parts of the world due to its low side effects.<sup>6</sup> The most common forms of drug preparation and consumption of herbal products can be listed as powder, pill, infusion, decoction, ointment, tincture, medicinal oil and scented oil.<sup>3</sup> During the preparation of the drug, the elements in the plant pass to the preparation.

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## Materials and Methods

Plant samples of sage (*Salvia officinalis* L.) and lemon balm (*Melissa officinalis* L.) which was cultivated in Yalova were used in the preparation of decoction samples.

### Preparation of plant samples

The leaves of medicinal sage and lemon balm were dried in the shade and herbal teas were obtained by decoction method. For this method, different amounts (2g and 3g) and different periods (5 min and 10 min waiting) were applied (Table 1). At the end of the application, the tea samples were filtered and stored in a refrigerator at 4°C until analyzed.

### Al, Ni and Co analyzes

Decoction samples digested with 5 mL Suprapur (Merck, Darmstadt, Germany) nitric acid (HNO<sub>3</sub>), 2 ml hydrochloric acid (Merck, Darmstadt, Germany) and 3 ml ultrapure water (Direct-Q; Millipore, Darmstadt, Germany) in the Teflon tubes using a microwave digestion system (Start D; Milestone, Maryland).

The digested samples diluted with ultrapure water to the total volume of 20 ml in a 50 ml polypropylene tube. Al, Ni and Co standards (Multi-Element Standard Chem-Lab, Zedelgem, Belgium) used for calibrations. 11-point calibration curve (0.5–1000 ppb) was used to measure the level of each element.

**Table 1:** Preparation of decoction samples of lemon balm and sage

Decoction Samples	Amount of samples (g)	Waiting time (min)
D1	2	5
D2	3	10
D3	2	5
D4	3	10

The  $r^2$  values of the calibration curves of all parameters were calculated, and the minimum value was 0.9998. Al, Ni and Co were measured using inductively coupled plasma-mass spectrometry (ICP-MS, ICP-Qc, Thermo Scientific, USA). The results of these measurements showed that the relative standard deviations did not exceed 5%. Certified Reference Material (CRM-Seronorm Trace Elements Whole Blood L-2, Sero AS, Billingstad, Norway) was used for the validation method. Internal standard (Hf) was used to check the stability and sensitivity of the instrument.

### Statistics

All data was statistically analyzed using one-way ANOVA, and comparison of the means was carried out by Duncan's multiple range tests at a significance level of 0.05 and the data were given as the mean  $\pm$  standard error. The data matrix of

the plant samples and their decoction samples was obtained from hierarchical cluster analysis.

## Results and Discussion

In this study, the effects of different amounts and time application on the heavy metal contents such as Al, Ni, and Co which are found in the decoction samples of sage and lemon balm plants and affect human health were investigated. In both species, the highest amounts were Al, Ni and Co, respectively.

For sage decoction samples, the highest amount of Al was statistically highest in AD1 and AD4 samples at 331.8 and 319.6 ppb, respectively.

The highest Ni content was observed in the AD4 decoction sample. The amount of Co was found to be statistically insignificant in all decompression samples (Table 2).

**Table 2:** Heavy metal contents in sage and its decoction samples (ppb)

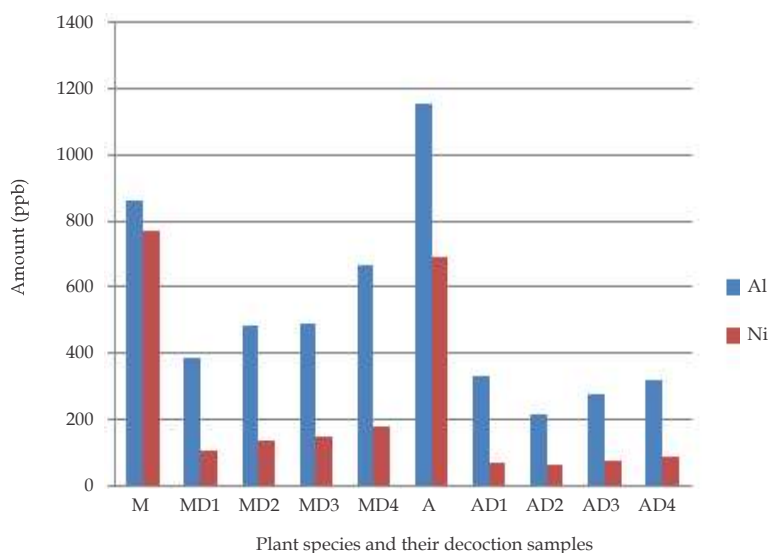
Sample	Al	Ni	Co
Dry sample (A)	1155.6	692.3	75.6
Decoction			
AD1	331.8	69.3	7.6
AD2	212.6	60.8	7.2
AD3	276.6	74.8	8.0
AD4	319.6	86.0	10.2

The highest Al and Ni content was found in the MD4 decoction samples. Co contents were reported to be statistically insignificant in decoction samples similar to sage (Table 3). However, the maximum amount of Co in the decoction samples of both species was observed in D4 decoction samples.

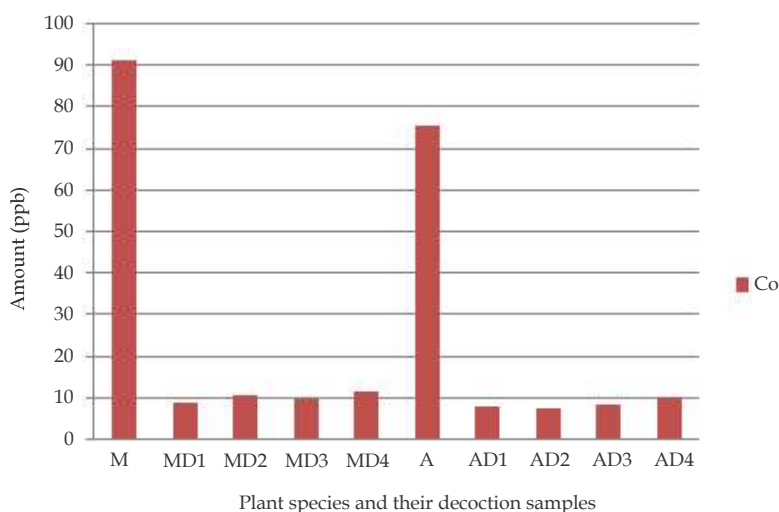
When the sage and lemon balm were compared, the amount of Al was observed in the highest sage with 1155.6 ppb in herba samples, but in all the decoction samples of the lemon balm plant (Fig. 1). The amount of Ni was determined both in herba samples and in the highest lemon balm plant in terms of decoction samples. When Fig. 2

**Table 3:** Heavy metal contents in lemon balm and its decoction samples (ppb)

Sample	Al	Ni	Co
Dry sample (M)	862.1	771.3	91.6
Decoction MD1	384.9	105.4	8.4
MD2	485.7	136.8	10.2
MD3	490.6	146.7	9.5
MD4	664.5	178.2	11.4



**Fig. 1:** Al and Ni distribution in lemon balm, sage and their decoction samples (ppb).



**Fig. 2:** Co distribution in lemon balm, sage and their decoction samples (ppb).

was examined, Co was also observed in both herba and decoction samples (MD4) of the highest lemon balm species.

Table 4 shows how many percent of the Al, Ni and Co micatars contained in the plant are transferred to the decoction samples. It was determined that Al was the most transitive element among the heavy metals

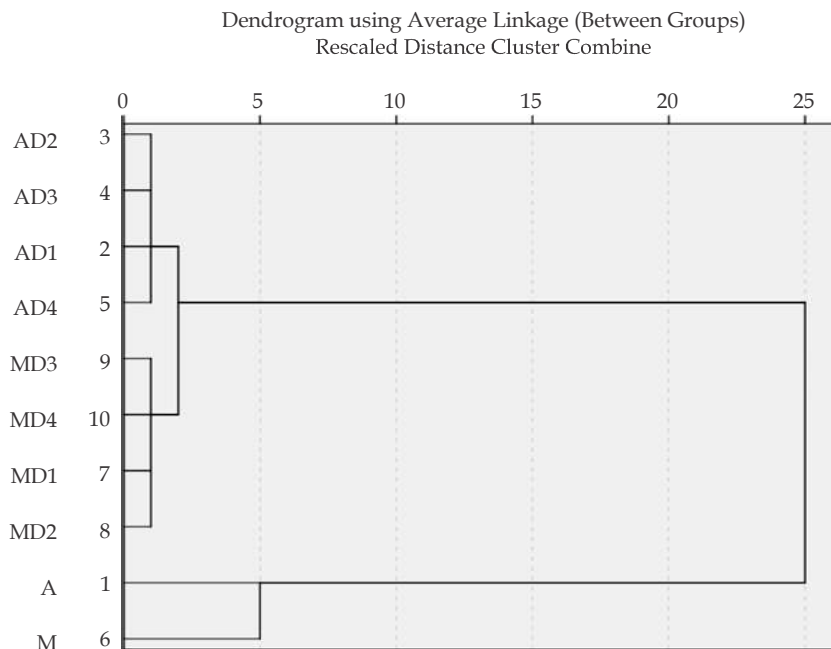
in both species (Average, M: 58.7% and A:24.7%). The highest Al transition to the decoction samples was observed in the D4 decoction application of lemon balm plant with 77.1%. This shows that the amount of transition is very high. This situation is important for human and public health. In terms of Co and Ni, Ni was found to be the highest element transition percentage (18.4%) in lemon balm and

**Table 4:** Percentage of the elements in their decoction samples compared to the plant species

	Al	Ni	Co
<b>Lemon Balm</b>			
MD1	44.7	13.7	9.1
MD2	56.3	17.7	11.2
MD3	56.9	19.0	10.4
MD4	77.1	23.1	12.4
Average	58.7	18.4	10.8
<b>Sage</b>			
AD1	28.7	7.2	10.1
AD2	18.4	6.3	9.6
AD3	23.9	7.8	10.5
AD4	27.7	8.9	13.5
Average	24.7	7.5	10.9

Co element (10.9%) in sage. However, in the sage and lemon balm Co element has been observed to have similar values on average (10.9% and 10.8%, respectively). When the effect of different plant sample and decoction time on the amount of Al, Ni and Co in the preparation of the decoction samples

were examined, it was found that the amount and transition time of these heavy elements increased as the amount and application time increased. However, it was observed that the amount of 2 g substance in sage was more effective than 5 min application (AD1) than 10 min application (AD2).



**Fig. 3:** Dendrogram of sage, lemon balm and their decoction samples (versus each of the four elements under study) obtained by hierarchical cluster analysis using square euclidean distance.

In the application of 3 gr, it was recorded that the amount of transition of the elements increased with increment time.

A hierarchical clustering by applying the Between-groups linkage method, which uses the squared euclidean distance as a similarity measure, was applied using the SPSS package. In dendrogram in Fig. 3, according to the elements tested, the decoction samples of sage were in the same group (Group 1), and the decoction samples of lemon balm were in the same group (Group 2), as well as dry samples of sage and lemon balm were in the same group (Groups 3). Group 1 was in the group close to Group 2 while Group 3 alone formed a separate entity from other groups.

### Conclusion

As a result, toxicological evaluation of such plants used for their therapeutic properties is important for human and public health.

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