HEAVY METAL CONTENT ANALYSIS IN SALVIA OFFICINALIS PLANTS BY GRAPHITE FURNANCE ATOMIC ABSORPTION SPECTROMETRY

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Sage-Salvia Officinalis este o plantă medicinală importantă, cu puternice proprietăți antioxidante, acordându-se atenție metodelor analitice pentru determinarea conținutului speciilor chimice anorganice şi organice din aceasta plantă. Printre acestea, spectrometria de absorbție atomică cu atomizare electrochimică este o tehnică puternică pentru determinarea constituenților metalici, incluzând aici şi metalele grele.

Probe de Salvie de pe Valea Prahovei - România au fost analizate în scopul stabilirii conținutului de cadmiu şi plumb. Soluțiile de probă au fost obținute folosind cuptorul cu microunde. Analizele de Cd şi Pb au arătat un conținut scăzut în aceste metale grele, observându-se o uşoară variabilitate. S-au luat în considerare particularitățile referitoare la diferitele părți ale plantelor.

Sage-Salvia Officinalis is an important medicinal plant with strong antioxidant properties and attention has been granted to analytical methods for the content determination of inorganic and organic chemical species from this plant. Among them, atomic absorption spectrometry with graphite furnace (GFAAS) is a powerful tool for the determination of metallic constituents, including heavy metals.

Sage- Salvia Officinalis samples, from Prahova Valley region - Romania, have been analyzed in order to establish the content of cadmium and lead. The samples have been digested by the microwave oven. Cd and Pb analyses showed a low content of these heavy metals, however a slight variability was observed. Particularities regarding plants parts were identified and taken into account.

Keywords: Salvia Officinalis, heavy metals, graphite furnace atomic absorption, spectrometry

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1. Introduction

Common Sage (*Salvia Officinalis*) is one of the most used herbal remedy in natural medicine, which is becoming more popular worldwide. The name of the genus, *Salvia*, is derived from the Latin *salvere* (to heal, to recover, to save) in reference to the multi-curative properties of this plant. Thus, Sage plant has anti-inflammatory, antibiotic, antispasmodic, antimicrobial, astringent and anti-perspiration properties, being used as domestic remedy for many diseases since olden times. This plant is known also as having the ability to rejuvenate cells and restores memory in old age. It is native to the Mediterranean region and is also widely used as food flavoring, having a slight peppery flavour [1]. In the same time, this plant possesses strong antioxidant properties, many pharmaceutical products containing different compounds extracted from Salvia Officinalis, especially essential oils. Salvia plants can be either naturally procured or from free market, as all herbal remedies. Among the factors affecting the healing properties of Salvia Officinalis are: the environment in which the plants grow, the presence of contaminants (like heavy metals) having a major negative influence, present even in very low concentration.

There are multiple sources of environmental contamination: mining, chemical industry, waste incineration, exhausts gases and the plants are able to absorb cadmium and lead ions and their complexes, too. It is also known, that it can not deal with environmental pollution with heavy metals without addressing these metals accumulation in plants, being equally impossible to tackle this without addressing heavy metal contamination chain ecosystem. Because of this, any heavy metal (especially Cd and Pb) accumulated by Salvia plants would increase the possibility of heavy metal toxicity to consumers.

Since medicinal plants, so any Sage, can be purchased "over the counter," and are not legally regulated from the point of view of contaminants, people are becoming more aware of the risks associated with the occurrence of heavy metals either in Salvia Officinalis plants or in its products (pharmaceutical, culinary and cosmetics). Therefore, attention has been granted to the development of analytical methods for the determination of the chemical species from Salvia Officinalis, either inorganic or organic chemical species[1-6]. Among advanced analytical techniques, atomic absorption spectrometry with electrothermal atomization (GFAAS) is a very sensitive and selective method and is becoming the technique of choice in the contamination control with heavy metals of a large variety of natural products.

In the present study, GFAAS method has been applied to determine the cadmium and lead content of Salvia samples collected from Prahova Valley region-Romania. Particularities regarding plants parts and age have been identified and taken into account.

2. Experimental

Chemicals

All the applied chemicals were of high purity grade. Certified stock standard solution 1000 mg/L of cadmium and lead were purchased from Fluka and they were diluted with bidistilled water as necessary to obtain working standards solutions. Nitric acid (65% w/v) was obtained from Riedel de Haen and hydrogenperoxide 30% was from Fluka. Because accurate trace levels analysis (µg/L) can be obtained only by prevention of element contamination, all stages of sample preparation and analysis were carried out in a clean environment. All the sample containers, glassware and autosampler plastic cups were cleaned with nitric acid 20% for 24 hours and then rinsed abundantly with bidistilled water prior use. The washing solution employed to clean the capillary was a solution containing HNO_3 1.0 %.

Sampling

Ten selected Salvia plants were sampled in June 2009 from 5 different points of Prahova Valley (located as shown in the Figure 1), each at a distance greater than 500 m from the main road (not to be influenced by traffic pollution). In order to obtain a representative sample, two annual plants were harvested from each sampling point using new plastic bags. A sample from the herbal shop was also analyzed. For the digestion of plants material, a wet decomposition procedure using the Berghof speed-wave MWS-2 microwave pressure digestion system was chosen.

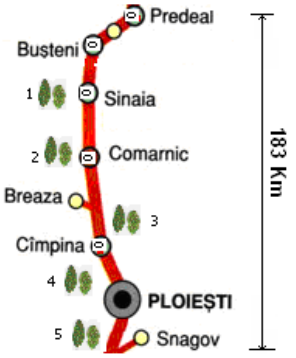

Fig.1. Map of Prahova Valley showing the five points for Salvia sampling

Sample preparation for analysis was achieved as follows: Salvia plants were thoroughly washed with tape water, followed by distilled water and different plant parts (roots, above-ground parts of the plant and flowers) were separated and oven dried at 80 ^{0} C temperatures for 24 hours (until the dry weight was constant). The different plant parts dried then were smashed and powdered in, 0.2-0.3 grams of each powdered sample being digested using the pressure microwave system with a mixture of 3 mL of nitric acid 65 % and 2 mL of hydrogen peroxide 30% in

DAP-60K Teflon reaction vessels. The mixture is stirred thoroughly with a clean glass bar and then waited at least 20 min before closing the vessel. The heating of the Teflon vessels in the microwave oven was achieved with a temperature program set in three steps, optimized for the samples under study (*Table 1).*

Table 1

The microwave oven program optimized for Salvia samples digestion

Step	1	2	3
T [°C]	145	180	100
Power* [%]	75	90	40
Time [min]	5	10	10

In order to avoid foaming and splashing, after the end of the temperature program, the digestion vessels have to be cooled down to room temperature waiting about 40 min before opening the vessels cover. Because during the digestion process produces a large amount of gas, the vessels must be carefully open in a fume hood, and the operator must wear protective equipment (goggles, gloves and lab coat). The resulting clear sample solution is transferred into a 50 mL measuring flask and completed with bidistilled water. A blank- sample is obtained in the same way as a real sample.

Instrumentation

Cadmium and lead were determined by GFAAS. A highly sensitive atomic absorption spectrometer Analytic Jena 650 ZEENIT equipped with Zeeman and deuterium lamp for continuous background correction was used. The instrument was also provided with hollow cathode lamps for Cd and Pb, operated as recommended by the manufacturer at 15, and 20 mA respectively, and with an autosampler model MPE 60 z. For graphite furnace action was used argon as inert gas. The absorbance measurements were performed using the spectral lines of Cd at 228.8 nm and of Pb at 283.3 nm and the graphite furnaces in form of wall tub fitted up with its own control unit were chosen. Peak height absorbance measurements were used for both element determinations.

The spectrometer parameters were set at the beginning of the measurements to the recommended ones by user guide, and after preliminary tests, they were optimized for cadmium analysis.

The employed graphite furnace was wall tub fitted up with its own control unit, the temperature program being optimized as shown in *Table 2*.

Table 2

Heating programme parameters of graphite furnace for Cd and Pb analysis

Operation	Cd			Pb		
	T (^{o}C)	Ramp (^{o}C/s)	Time (s)	T (^{o}C)	Ramp (^{o} C/s)	Time (s)
Drying 1	90	20	10	90	20	13.5
Drying 2	105	1	15	105	1	25
Draying 3	120	2	10	120	3	15

Ashing	300	250	10	500	250	12
Autozero	300	0	4	500	0	4
Atomisation	900	1500	3	1500	1400	4.7
Cleaning	2100	500	4	2100	500	5

3. Results and Discussions

The GFAAS method was optimized for low trace Cd and Pb determinations. The detection limit (LOD), defined as the concentration corresponding to three times the average of standard deviation of 21 blanks was for Cd 0.44 μg/kg and 1.37 μg/kg for Pb. Quantification limit (LOQ) for Cd was 0.88 μg/kg and 2.64 for Pb, both LOD and LOQ being calculated in accordance with reference 7.

The linearity of calibration curves for the expected range concentration of Cd and Pb in plant samples was verified, and as expected, a very good linearity was obtained for Cd in the range of 0-1.2 μg/L ($R^2 = 0.9996$) and for Pb 0-20 μg/L ($R^2 = 0.9989$).

The mean of Cd and Pb contents obtained from three replicates of various samples of Salvia Officinalis (each sample was obtained from two plants collected) are shown in *Table 3*. According to these data, a low content of these metals has been found in all analysed samples, however a slight variability has been observed. The higher Cd and Pb concentrations were found in sage samples collected from point 2, for all part plants, especially for roots samples, but still below their toxic level. One possible explanation may be that in this location for many years a cement factory was operated. Great differences regarding the content of heavy metals between the aerial parts of the plants were not observed.

Table 3 shows also that Cd and Pb were accumulated firstly in the roots, a higher content of these metals being recorded for this part plant in comparison with the aerial parts for all analysed samples.

The small values of Cd and Pb were found for the sample Sage purchased from herbal shop (2.6 μg/kg and 3.83 μg/kg, respectively) and analyzed in the same way as all other samples.

Table 3

Concentration of heavy metals (ppb) in *Saliva Officials samples*

Collection point	Part plant	*Cd, μg/kg SD	*Pb, μg/kg SD
1	Roots	3.7 ± 1.29	5.16 ± 2.22
	Aerial	2.6 ± 1.56	3.58 ± 0.97
	Flowers	3.0 ± 0.56	2.55 ± 1.47
2	Roots	10.7 ± 1.43	12.94 ± 2.63
	Aerial	8.4 ± 0.74	11.75 ± 1.38
	Flowers	8.9 ± 1.65	10.34 ± 2,64
3	Roots	2.9 ± 1.17	2.99 ± 1.48
	Aerial	1.9 ± 1.34	2.82 ± 1.73
	Flowers	1.2 ± 0.92	2.65 ± 2.38

4	Roots	8.7 ± 1.21	7.35 ± 1.59
	Aerial	6.9 ± 1.56	5.73 ± 2.41
	Flowers	6.1 ± 1,02	4.91 ± 2.72
5	Roots	6.8 ± 1.63	8.73 ± 2.11
	Aerial	3.6 ± 2.05	7.37 ± 1;48
	Flowers	4.3 ± 1.27	7.85 ± 0.95
Herbs shop	As presented	2.6 ± 2.31	3.83 ± 1.44

*Data as mean ± standard deviation

Even if the aerial parts of Salvia Officinalis are used more than the roots (used in very special cases, such as cardiac edema and Alzheimer's disease), much attention should be given to the place and to the plants sampling procedure. A special importance should also be given on the storage of plants and even to water quality for Sage tea and its products.

4. Conclusions

Hazardous heavy metals (cadmium and lead) content in Salvia Officinalis samples were determined by graphite furnace atomic absorption spectrometry, using a considerably simple sample preparation procedure. The analyzed plants sampled from the specified locations have an insignificant amount of Cd and Pb, although a relative higher content was observed in a contaminated environment (point 2), but still bellow the toxic level. Therefore, it is appropriate to analyze the content of heavy metal in herbal plants in order to decrease the possibility of heavy metal toxicity to consumers. On the other hand, this study can also lead to the assessment of the impact of environmental pollution with heavy metals on the composition of different herbs.

Due to its possibility to accumulate heavy metals, it could not ignore the potential of Salvia Officinalis to be used as a detoxifier for heavy metal poisoning cases, an option which was not taken into account until now.

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