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Original Research

Heavy Metals Content in Leaves and Extracts of Wild-Growing *Salvia Officinalis* from Montenegro

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Abstract

Salvia officinalis L. (Dalmatian sage) is used traditionally in medicine as herbal tea and as a tonic, antiseptic and for inflammations and infections in the mouth. Also, sage is widely used as food flavouring, either as dried leaves or as the essential oil or oleoresin. Thus, any heavy metal accumulation by this plant would increase the possibility of heavy metal toxicity to consumers.

In Montenegro, sage and some other aromatic herbs grow wild in close proximity to major roads. This study aims to establish the impact of pollution on the composition of sage leaves and this first investigation acts as a platform from which further work will follow.

The total contents of Pb, Cd, Cu and Zn in *Salvia officinalis* leaves and in essential oil, solvent extract, tea brew and tea infusion at selected distances from a heavy metal pollution source was examined.

Generally, it was found that the concentration of heavy metals decreases at 50 m, i.e. 100 m from the edge of a major road. Therefore the impact of road traffic through the pollution of aromatic herbs was noted. The concentration of hazardous heavy metals Pb and Cd in all investigated sage extracts was bellow detection limits of the analytical technique used while the solubility of heavy metals Zn and Cu was the highest in tea infusion extract.

Keywords: *Salvia officinalis*, road pollution, extracts, tea, heavy metals

Introduction

The genus *Salvia* is one of the largest members of the family *Lamiaceae* (subfamily Nepetoideae) comprising nearly 900 species spread widely throughout the world [1]. *Salvia officinalis* L. (Dalmatian sage) is a perennial subshrub that came originally from the area around the Mediterranean but it has been cultivated elsewhere in the world for culinary and medical purposes [2].

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Sage has great economic significance. Many Mediterranean countries where it grows gain substantially from the production and export of sage. It is used in the meat industry. It is also used as an ingredient in several phytopreparations, for gargling, and it has an antibacterial effect. Herbal tea based on sage is also used for body strengthening [3]. Sage essential oil and oleoresins are used in the food, beverage and perfume industries, with several therapeutic applications [4].

Heavy metals are naturally present in the environment. However, the dynamic development of industry and motorization, as well as the continuing over-intensive use of various chemical compounds in agriculture, cause levels of toxic heavy metals in the environment to constantly be on the increase. Lead and cadmium are on the list of the most dangerous metals that cause acute and chronic environmental contamination. Copper and zinc are among physiological elements essential for the correct functioning of living organisms but, in high concentration, can be also harmful to organisms [5].

The human body requires both metallic and non-metallic elements for healthy growth and development within certain permissible limits. Using herbs and their extracts in medical treatment of various illnesses, besides the sought-after pharmacological effect, could be dangerous because of heavy metals and other impurities. For this reasons it is important to control the level of contaminants in medicinal raw materials [6]. The analytical determination of metals in medicinal plants is part of quality control in order to establish their purity, safety and efficacy. Several studies have been carried out on the impact of traffic on metal concentration levels in road dust, adjacent soils and food crops [7-15].

To the best of our knowledge this is the first study to investigate the metal content of aromatic herbs and its extracts related to the vicinity of a major road in Montenegro.

The aim of this paper was to determine the influence of road vicinity on the content of heavy metals (Pb, Cd, Cu, Zn) in wild-growing Salvia officinalis L. leaves, essential oil, oleoresin and extracts, before and after flowering period, and the possibility of the utilization of herbs from the chosen collection site with regard to heavy metal content.

Experimental Procedure

Collection of Herb Material and Description of the Study Area

Leaves of wild-growing sage (*Salvia officinalis* L., Lamiaceae) were collected manually previous to (in March) and after (in July) the flowering stage in 2007 in Dobra voda (at an altitude of 100 m), the coastal part of

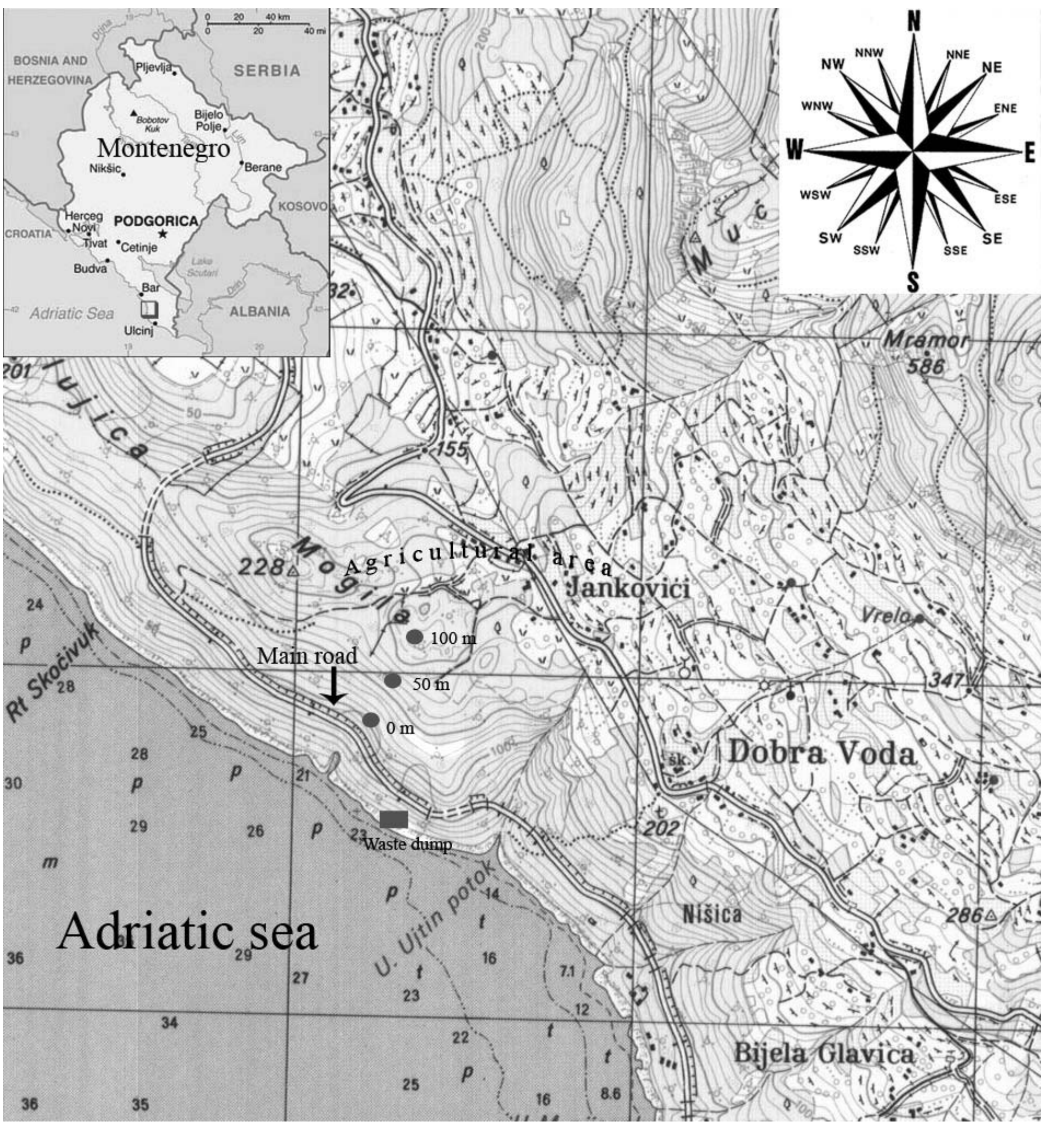

Fig. 1. Map of the investigation site.

Table 1. Heavy metals content (mg/kg) in samples of sage leaves by FAAS method.

	Before flowering period			**After flowering period**		
	0 m from the road	**50 m** from the road	**100 m** from the road	**0 m** from the road	**50 m** from the road	**100 m** from the road
	(mg/kg)[1]					
Pb	10.42 ± 0.10	6.90 ± 0.04	6.48 ± 0.04	4.19 ± 0.02	3.23 ± 0.02	2.16 ± 0.01
Cd	0.068 ± 0.001	0.062 ± 0.001	0.043 ± 0.001	<0.001	<0.001	<0.001
Cu	12.29 ± 0.20	10.73 ± 0.10	9.25 ± 0.09	13.89 ± 0.20	11.49 ± 0.20	9.60 ± 0.15
Zn	66.62 ± 0.40	51.12 ± 0.40	40.18 ± 0.35	13.64 ± 0.15	13.29 ± 0.15	13.13 ± 0.10

[1]Means ± S.D. of five measurements.

Montenegro (near Bar). The leaves were collected from the different collection sites with regard to the vicinity of the road, at the edge of the road and at a distance of 50 m and 100 m from the major road, at its northern part (Fig. 1). The leaves were dried at room temperature and stored in paper bags until further analysis. The identity of plant specimen was confirmed at the Department of Biology, Faculty of Natural Sciences and Mathematics, University of Montenegro, where the voucher specimen was deposited.

The surveyed major road has high traffic density, especially in the summer during tourist season. Additionally, in the vicinity of the studied area, at south part of the road at distance of 150 m, there is a site with unsorted waste which is very often self-combusted. The prevailing winds in this area are usually NE and ENE (over 45%).

Preparation of Herb Material

Herb material was milled in a domestic blender (Zepter MixSy, type VG-022-K) and, after sieving (laboratory Erweka sieves), a sample with a mean particle diameter size of 0.7 mm was obtained. A prepared batch was kept in a hermetically sealed bag and stored at 8ºC for 2 days before use, in order to avoid losses of volatile compounds.

Determination of Heavy Metal Content in the Herb Material

A dry ashing method was used for destruction of organic matter to determine all of the heavy metals in teas. The crucibles with herb samples (8 g) were partially covered and carefully dried on a burner (~10 minutes). Then the samples were charred on a hot plate (~2 h) and ashed in a muffle furnace at 450°C for 12 h. After ashing, the ash was dissolved with 10% (v/v) HCl and then completed to a definite volume with 1% (v/v) HCl for analysis by flame AAS, Perkin/Elmer 4000.

Essential Oil Preparation

Herb material (20 g) was submitted to hydrodistillation in a Clevenger-type apparatus for 2 hours according to Yugoslav Pharmacopoeia IV. The obtained oil was dried over anhydrous sodium sulphate, measured, poured in hermetically sealed dark-glass containers and stored in a freezer at -4°C until analyzed by atomic absorption spectrophotometer. Obtained oil was acidified with 1% (v/v) HNO_3 for analysis by flame AAS, Perkin/Elmer 4000.

Oleoresin Preparation

The solvent extractions were carried out in a Soxhlet apparatus using petrol-ether. The Soxhlet thimble was charged with 12 g of plant material. After a 5 h extraction, the solvent (approximately 250 ml) was removed by distillation. Obtained extracts were acidified with 1% (v/v) HNO_3 for analysis by flame AAS, Perkin/Elmer 4000.

Plant Extract Preparation

Prepared herb material was extracted using water by two different methods commonly used at home: brew and infusion method. In the first method, 2 g of the dry herb was left to boil in 100 ml of distilled water for 5 min in a glass beaker. In the second method, 2 g of the dry sample was immersed in 100 ml of recently boiled distilled water for 5 min. The liquid extracts in the two procedures were held at room temperature and then filtrated separately. Obtained filtrates were analyzed by flame AAS, Perkin/Elmer 4000.

Chemical Investigation

Heavy metals in all prepared samples were determined by flame atomic absorption method (FAAS). A Perkin/Elmer model 4000 atomic absorption spectrophotometer with flame atomization (acetylene-air) was used for measurement of the metals. Maximum absorbance was obtained by adjusting the cathode lamps at specific slit width and definite wavelengths as recommended by the method as follows: 0.7, 228.8 nm (Cd); 0.7, 283.3 nm (Pb); 0.7, 213.9 nm (Zn) and 0.7, 324.8 nm (Cu).

Statistical Analysis

The results were statistically analyzed and the significance of differences among means was determined using Student's t-test by the Mathcad 7.0 program.

Results and Discussion

Heavy metals contents in medicinal herbs depend on climatic factors, plant species, vegetation period, air pollution, and other environmental factors [16]. Previously conducted investigations demonstrated that the distribution of the heavy metals among plant organs was selective and depended on the part of the plant, surface characteristics of the plant organ, and the element that was examined. It was found, by Angelova et al. [17], that Pb, Zn, and Cd distribution in *Salvia officinalis* decreased in the order: leaves > inflorescences > roots > stems and for Cu in the order: leaves > roots > inflorescences > stems. Because of that, in our paper we investigate the sage leaves, as part of the herb with the major heavy metal content and at the same time is mostly used.

The concentration of heavy metals in leaves and differently obtained extracts of the *Salvia officinalis*, at distances of 0 m, 50 m and 100 m from the major road are investigated in this paper. The results of total content of the studied heavy metals in *Salvia officinalis* leaves, with regard to different vegetation period and major road vicinity, are given in Table 1.

The most abundant metal in sage leaves harvested before flowering period was Zn, and its content decreases in samples at distances of 50 m (51.12 mg/kg) and 100 m (40.18 mg/kg) compared with samples at a distance of 0 m from the road (66.62 mg/kg). The same trend is present in concentrations of Cu – 12.29 mg/kg near the road, 10.73 mg/kg at 50 m and 9.25 mg/kg at 100 m from the road.

In the previous study [18] it was found that the mean Zn concentration in investigated aromatic herbs, in unpolluted areas of Montenegro, is 11.23-25.12 mg/kg, while the concentration of Cu in the same study was 6.79-9.46 mg/kg. The reason for high concentrations of Zn, compared to this study, can be explained by the use of zinc in lubricating oil, tires and accumulators. The source of Cu from traffic has been reported to originate from corrosion of metallic parts of vehicles.

The concentration of Pb found in sage leaves at a distance of 50 m (6.90 mg/kg) and 100 m (6.48 mg/kg) from the road, in both herb samples, decreases in comparison with the herb at the edge of the road (10.42 mg/kg).

The similar trend, with regard to descending content of metal compared to road vicinity, was monitored for samples of sage leaves harvested after the flowering period. Thus, the concentration of Zn decreases from 13.64 mg/kg (near the road), through 13.29 mg/kg (50 m from the road) to 13.13 mg/kg (100 m from the road). The same trend is present in concentrations of Cu – 13.89 mg/kg near the road, 11.49 mg/kg at 50 m and 9.60 mg/kg at 100 m from the road, while the concentration of Pb in the sage leaves near the road was 4.19 mg/kg, 3.23 mg/kg at 50 m and 2.16 mg/kg at 100 m from the road. Cd was not found in the herb samples studied.

From the results in Table 1 it can be concluded that the examined herb from different vegetation periods has different heavy metal concentrations.

Thus, for Pb, Cd and Zn a lower concentration (compared to herb harvested in March, before flowering period) is observed in the herb harvested in July (after flowering period), though there is a higher traffic density in that period on the investigated road. This can be explained by the fact that the harvested leaves in July are partly from the novel herbs, without sufficient time for extensive heavy metals accumulation from the air and soil.

Also, this specific region has a very hot summer period with extremely high temperature, so the lower metals level is partly a result of inhibited movement of the metal through the plant conductive system from the dry soil.

The enhanced level of Cu in these samples, on the other hand, could be explained by the vicinity of orchards and vineyards, in which the utilization of Bordeaux mixture (plant inorganic fungicide based on copper sulfate pentahydrate) in the previous months is extensive. Due to the prevailing northeast wind, some of that material reaches the investigated sage leaves' surface.

As a result of the lack of information and regulations about acceptable content of heavy metals in medicinal and aromatic herbs, it was difficult to accurately comment on the obtained results. Commission Regulation EC/466/2001 (78/2005) sets limits for Lead and Cadmium in leafy vegetables and fresh herbs (Pb 0.3 mg/kg; Cd 0.05 mg/kg) [19]. According to this, samples under investigation were above permitted limits (Pb in all samples and concentration of Cd in herb samples before flowering period). The Ph. Eur. Monograph "Fucus" is the only monograph of the providing limits for heavy metals in an herbal drug (mg/kg: Lead 5, Cadmium 4, Mercury 0.1, Arsenic 90) [20]. Data yielding of a heavy metal screening (more than 12.000 samples tested) show that the recommended limits are not generally feasible [21]. The German Pharmaceutical Manufacturers Association (BAH) therefore made a proposal for general limits derived from a statistical evaluation of a data bank compiled by its contaminant working group and limits for heavy metals (mg/kg: Lead 10, Cadmium 1, Mercury 0.1, Arsenic 5, Nickel 10, Copper 40) [22].

The heavy metal content in the samples under investigation was recorded at different levels, mostly under previously mentioned limits for heavy metals in an herbal drug (except Pb near the road – 10.42 mg/kg).

The general view on heavy metals content in the sage essential oil (Table 2) showed that the concentration of toxic heavy metals Pb and Cd, as well as Zn, was bellow detection limits of used analytical technique. Regarding Cu content, as the distance of herb sample from the road increases, its concentration in sage essential oil decreases from 0.0091 mg/kg (near the road), through 0.0073 mg/kg (50 m from the road) to 0.0058 mg/kg (100 m from the road).

The obtained results were compared with maximum permissible concentration approved in plant tissue in Bulgaria, because of the deficiency of official data in our country. Thus, the maximum permissible concentrations of Pb and Cu in the plant essential oil are 0.10 mg/kg, while the maximum permissible concentrations of Zn and Cd in the essential oil are 10.00 mg/kg and 0.05 mg/kg, respectively. The concentration of heavy metals in investigated essential oil from sage leaves is below indicated values.

Table 2. Heavy metals content (mg/kg) in samples of sage leaf essential oil by FAAS method.

	Before flowering period			After flowering period		
	0 m from the road	**50 m** from the road	**100 m** from the road	**0 m** from the road	**50 m** from the road	**100 m** from the road
	(mg/kg)[1]					
Pb	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
Cd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cu	0.0091±0.002	0.0073±0.002	0.0058±0.001	0.097±0.002	0.080±0.002	0.067±0.001
Zn	<0.06	<0.06	<0.06	0.018	0.008	0.006

[1]Means ± S.D. of five measurements.

Table 3. Heavy metals content (mg/kg) in samples of sage leaf oleoresin by FAAS method.

	Before flowering period			After flowering period		
	0 m from the road	**50 m** from the road	**100 m** from the road	**0 m** from the road	**50 m** from the road	**100 m** from the road
	(mg/kg)[1]					
Pb	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
Cd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cu	0.51 ± 0.02	0.33 ± 0.02	0.32 ± 0.02	0.74 ± 0.04	0.61 ± 0.04	0.48 ± 0.02
Zn	16.17 ± 0.40	8.65 ± 0.20	4.38 ± 0.10	3.48 ± 0.09	3.38 ± 0.10	2.72 ± 0.09

[1]Means ± S.D. of five measurements.

Table 4. Heavy metals content (mg/kg) in samples of sage leaf brew extract by FAAS method.

	Before flowering period			After flowering period		
	0 m from the road	**50 m** from the road	**100 m** from the road	**0 m** from the road	**50 m** from the road	**100 m** from the road
	(mg/kg)[1]					
Pb	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
Cd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cu	9.25 ± 0.20	7.63 ± 0.20	6.57 ± 0.15	9.86 ± 0.20	8.16 ± 0.20	6.82 ± 0.09
Zn	11.56 ± 0.40	7.65 ± 0.20	5.16 ± 0.20	2.31 ± 0.04	1.86 ± 0.04	1.58 ± 0.04

[1]Means ± S.D. of five measurements.

Table 5. Heavy metals content (mg/kg) in samples of sage leaf infusion extract by FAAS method.

	Before flowering period			After flowering period		
	0 m from the road	**50 m** from the road	**100 m** from the road	**0 m** from the road	**50 m** from the road	**100 m** from the road
	(mg/kg)[1]					
Pb	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
Cd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cu	10.85 ± 0.30	8.34 ± 0.20	7.14 ± 0.15	12.22 ± 0.40	8.96 ± 0.30	7.41 ± 0.20
Zn	16.10 ± 0.40	10.72 ± 0.30	7.20 ± 0.20	3.27 ± 0.10	2.66 ± 0.07	2.23 ± 0.06

[1]Means ± S.D. of five measurements.

Table 6. Heavy metals solubility (%) in sage leaf essential oil, oleoresin, brew and infusion.

Heavy metal	Essential oil	Oleoresin	Tea brew	Tea infusion
	Solubility (%)			
Pb	-	-	-	-
Cd	-	-	-	-
Cu	0.69-0.74	3.13-5.31	70.99-71.04	77.14-88.26
Zn	0.00-0.13	10.90-25.51	12.03-17.35	16.99-24.17

Toxic heavy metals Pb and Cd concentrations were too small to be detected using the available analytical technique in oleoresin, brew and infusion extract, while the concentration of Zn detected in oleoresin is relatively high (up to 16.17mg/kg) compared to its concentration in other investigated extracts (Tables 3, 4 and 5).

The concentration of extracted Cu in obtained oleoresin samples is rather low (0.32-0.51 mg/kg). On the other hand, the content of Cu in brew and infusion extract is relatively high (Tables 3 and 4), from 6.57 mg/kg to 9.25 mg/kg and 7.14 mg/kg to 10.85 mg/kg, respectively.

It can be noticed that there is significant decrease in Zn and Pb concentration in brew extract compared with their corresponding values in infusion. This drop could be attributed to chelation of these metals with tannic acid and tannins which exudate during the boiling of sage particles. Precipitation of these chelates leads to a decrease of metal concentration in the brew extract [24].

Solubility of the studied metals, calculated as a ratio between essential oil, oleoresin, brew and infusion extractable metal and its total content is given in Table 6.

Toxic heavy metals Pb and Cd concentrations were too small to be detected in all extracts, using the available analytical technique. Solubility of Cu in essential oil was rather low in range (0.69-0.74%) in oleoresin (3.13-5.31%), while in brew and infusion solubility of Cu it was (70.99-71.04%) and (77.14-88.26%), respectively. Solubility of Zn ranged from 0.00% to 0.13% in essential oil, while its solubility was 10.90-25.51%, 12.03-17.35% and 16.99-24.17%, in oleoresin, tea brew and tea infusion extract, respectively.

These results show that a relatively small amount of heavy metals were extracted from the sage leaves (except for Cu in tea brew and tea infusion), while the higher content remains insoluble in the solid particles during extract preparation. Therefor, disposal of the residual herb particles, after extractions, should be done properly, in order to avoid spreading of these heavy metals in the environment.

Conclusion

Heavy metals are present in spices and medicinal plants at different concentrations, which, in some cases, exceeded the permissible levels. This could be attributed to contamination from traffic and industrial activities, as well as utilization of various fertilizers and pesticides.

In general, the concentration of heavy metals is closely related to the distance from roadways and significantly lowers when the sage sample is obtained 50-100 m from the road. The obtained results show that *Salvia officinalis* is subject to contamination of the environment by heavy metals, but not as expected (due to the vicinity of major road and trash dump) because of prevailing wind direction in the investigated area and declining consumption of Pb-based gasoline in this region.

Summarizing the obtained results shows that toxic metals Pb and Cd were insoluble in all extracts. Extraction of herb material by water shows higher solubility of Cu and Zn in tea brew extract compared to tea infusion extract. Because of the lack of regulations about acceptable content of heavy metals in medicinal and aromatic herbs extracts, the maximum allowable and safe concentration of each metal is urgently needed.

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