

Essential Oil Variation and Trace Metals Content in Garden Sage (*Salvia officinalis* L.) Grown at Different Environmental Conditions

Mohammed Sanad Abu Darwish¹

¹ Department of Essential & Applied Sciences, Shoubak University College, Al-Balqa Applied University, Shoubak, Jordan

Correspondence: Mohammed Sanad Abu Darwish, Department of Essential & Applied Sciences, Shoubak University College, Al-Balqa Applied University, Shoubak, Jordan. Tel: 962-795-171-140. E-mail: maa973@yahoo.com

Received: June 25, 2013 Accepted: July 26, 2013 Online Published: February 15, 2014

doi:10.5539/jas.v6n3p209

URL: <http://dx.doi.org/10.5539/jas.v6n3p209>

Abstract

This study was conducted to determine the essential oil variation and concentrations of certain trace metals (Co, Cu, Fe, Mn and Zn, Pb, Cd) in the sage plant (*Salvia officinalis* L.) grown in their different geographic natural environment. The samples of *Salvia officinalis* were collected from Jeresh and Ajloun, Ma'an and Tafilah, and Amman located in their natural original regions in the north, south, and middle locations of Jordan, respectively. The results showed a wide variation of essential oil contents among *S. officinalis* grown in different variable natural environment. The range varied from 0.87% in Amman to 2.8% in Jarash. In general, the oil content in *S. officinalis* grown in the north regions was higher than recorded in the middle and south regions. The essential oil content in *S. officinalis* grown in Jarash and Ma'an were higher than recorded in other investigated growth regions. Trace metal concentrations in all investigated samples were varied. The most toxic trace metals Co, Pb, and Cd were not detectable in all studied samples. Fe metal recorded the highest concentration which varied from 834.5 mg/kg in Ajloun to 1743 mg/kg in Ma'an. Cu recorded the lowest mean levels among all detected metals and varied from 6.60 mg/kg in Amman to 9.25 mg/kg in Ajloun. The highest mean levels of Mn were recorded in the southern regions in Tafilah and Ma'an (53.7 and 50.4 mg/kg, respectively), while the lowest was recorded in the middle region in Amman (26.10 mg/kg). Zn concentration varied from 27.80 mg/kg in Ma'an to 42.72 mg/kg in Tafillah. All of detected metals were within the range of permissible limit for medicinal plants and lower than that detected in *S. officinalis* originated from other local and global habitats. The essential oil and trace metals contents in *S. officinalis* were mainly affected by variable natural climatic conditions. Moreover, the current study showed that *S. officinalis* grown in some locations of Jordan are characterized by low trace metals contents and can safely be used for pharmaceutical and edible purposes without any hazardous effect on human health.

Keywords: essential oil, Jordan, sage, trace metals

1. Introduction

Garden sage (*Salvia officinalis* L.) is a perennial medicinal plant with woody stems, and grayish leaves belonging to the family Lamiaceae that comprises over 900 species all over the world. It's native to the Mediterranean region in which twenty species of *Salvia* were found grow wild in Jordan (Al-Eisawi, 1996). *S. officinalis* is a popular plant in Jordanian folk medicine and used for edible consumption and herbal remedy as a source of therapeutically active essential oil (Amr & Đorđević, 2000). Sage extracts, mostly essential oil, are also widely used in the drug, beverage, cosmetic and fragrance industries. Various studies have proved that the essential oil extracts of garden sage exhibited antimicrobial activities against some pathogenic microorganisms and food borne bacteria (Hayouni et al., 2008; Klaus et al., 2008). Moreover, the individual secondary metabolites of sage could exhibit various pharmacological activities such as anesthetic, antihistaminic, antirheumatic, diuretic, expectorant, insecticidal, purgative (monoterpenes); analgesic, antiarrhythmic, antiepileptic, spasmolytic, anthelmintic, antiinflammatory, antitumorous, hypotensive, and sedative (Velickovic et al., 2003).

Trace metals of *S. officinalis* have an important biological role in human, animal and plant health. Trace metals content in medicinal plant research conducting is of great interest because of their effect on the biological active compounds in medicinal plants and may cause serious effects on human health. Thus, the maximum permissible limits of toxic metals in medicinal plants such as cadmium and lead were reported by World Health Organization,

(2007). In Jordan, a recent study was conducted to determine Pb concentration in seventy nine popular samples of medicinal plants (Alomary et al., 2013), they found that Pb concentration in the most samples was higher than recommended values by World Health Organization. The mean Pb levels were ranged between 13.1 and 16.9 $\mu\text{g/g}$ in the most and less commonly used herbs (Alomary et al., 2013). In addition, many worldwide research studies have been conducted to evaluate the content of heavy metals in certain medicinal plants consumed by popular or used in pharmaceutical industries (Amr & Đorđević, 2000; Malencic et al., 2003; Chan, 2003; Angelova et al., 2005; Szentmihályi & Then, 2007; Blagojević et al., 2009; Abu-Darwish et al., 2010).

The content of essential oil and heavy metals in medicinal plants can be affected by environmental conditions (Maksimovic et al., 2007) and geochemical characteristics of soil or location in which the plant is grow (Chan, 2003; Blagojević et al., 2009; Abu-Darwish et al., 2010). Therefore, the present study was conducted to find out the effect of environmental conditions on the yield of essential oil and certain trace metal contents in *S. officinalis* grown naturally in different geographical locations of Jordan.

2. Materials and Methods

2.1 Plant Material

Aerial parts of *S. officinalis* were collected in vegetative period during April, in five localities of Jordan: Ma'an (South Jordan); Tafillah (South Jordan); Jerash (North Jordan); S5- Ajloun (North Jordan); and Amman (Middle Jordan). Voucher specimens were deposited at the Herbarium of Shoubak University College- Al-Balqa Applied University. The plant material was dried in draughty place at about 20°C, all specimens were identified on the basis of macroscopic characteristics by comparison with authentic sample and a voucher specimen was deposited at the Herbarium of Al-Shoubak University College. The dried samples of *S. officinalis* were separately crushed and mild into small pieces and sieved through (0.5 mm) mesh sieve.

2.2 Determination of Essential Oil

Essential oil contents were extracted from dried aerial parts of the all collected samples of Sage plant by the hydro-distillation method using a Clevenger-type apparatus (British Pharmacopeia, 1998) similar to (European Pharmacopeia, 2007), using 50 g of the dried mild and sieved plant and 500 mL of water in 1000 mL round bottomed flask. Distillation time was 2 h at a rate of 2-3 min^{-1} the values reported are the mean of at least three distillations and three replications for each specimen.

2.3 Determination of Heavy Metals Content in Sage Plant Samples

The content of Co, Cu, Fe, Mn and Zn, Pb, and Cd in particular sage samples were analyzed using Atomic Absorption Flame Emission Spectrophotometer Varian Spectro AA.200 as described by (Al-Alawi & Mandiwana, 2007). Cathode lamps used as radiation source. Air acetylene gas was used for all the experiments. The Absorption wavelength for the determination of each metal together with its liner working range and correlation coefficient of calibration graphs are given in Table 1. The plant samples were oven dried at 70°C for 24 h until the dry weight was constant. The dried samples were then ground and passed through a 0.2 mm plastic sieve. Then, 0.5 g of plant sample was wet digested with an Ultra-pure nitric acid (HNO_3 (10-15 mL) in a polyethylene test tube using a heating block digestion unit at 120°C. The final solution was filtered into a 25 or 50 mL volumetric flask through a 45- μm filter paper and diluted to the mark with ultra-pure water. Ultra-pure water was used for all dilutions and sample preparation. Analytical results have evaluated by statistical analysis system. The standard error values of the means were calculated to compare the site categories.

Table 1. Operating parameters for working elements

Elements	Wave length(nm)	Lamp intensity (mA)	Slit width(nm)	Correlation coefficient(r)
Pd	217.0	5 mA	0.2	0.998
Cd	228.8	4 mA	0.5	0.998
Zn	213.9	5 mA	1.0	0.998
Cu	234.7	4 mA	0.5	0.998
Fe	248.3	5 mA	0.2	0.998
Mn	279.5	5 mA	0.2	0.998

3. Results and Discussion

Means of essential oil contents in *S. officinalis* L. are listed in Table 2. The results revealed a wide variation in the values of essential oil yielded from sage plant grown in different environmental locations. The variation range of oil content varied from 0.87 to 2.80%, reaching minimum and maximum values in Amman and Jeresh located in the middle and northern regions of Jordan, respectively. The results showed that the oil content in all specimens of *S. officinalis*, except that cultivated in Amman (Middle of Jordan) were found to satisfy the requirements of Pharmacopoeias such as European Pharmacopoeia, which requires a yield of oil should be $\geq 1.2\%$ v/w. calculated with reference to the anhydrous (dried) drug (European Pharmacopoeia, 2007). In south regions, Essential oil was ranged from 1.8 to 2.2% in specimens collected from Tafillah and Ma'an respectively, while it was increased from 1.20 to 2.8% in the northern growth areas of Ajloun and Jeresh, respectively. These results were in agreement with the results obtained by Amr and Đorđević (2000), who found that *S. officinalis* collected during the vegetative period from some middle locations of Jordan, yielded 1.18-1.32%.

Table 2. Averages of ambient temperatures(°C), rainfall (mm), and relative humidity (%), and means of essential oils yield (%), and moisture content (%) of garden sage cultivated in different environmental regions in Jordan

Place of Sage growth	Altitude (m)	Ambient temp. min – max. (°C)	Seasonal means of rainfall (mm)	Relative humidity (%)	Moisture (%)	Oil (%)
Ajlune	800-1150	10.1 – 18.6	582.2	80.20	4.9%	1.20±0.17
Jarash	520-750	8.5 – 18.40	445.6	68.30	3.4%	2.80±0.06
Amman	700-1050	6.8 – 23.5	490.0	51.70	3.7%	0.87±0.12
Ma'an	1069-1100	20.0 – 32.0	44.20	57.80	6.1%	2.20±0.17
Tafelah	900-1140	11.8 – 23.4	237.6	23.30	5.2%	1.80±0.17

Table 3. Concentration of heavy metals (mg/kg) in Sage (*Saliva Officinalis*) in various locations in Jordan

Location	mg/kg						
	Co*	Cu*	Fe*	Mn	Zn	Pb*	Cd*
Ajlune	<IDL	9.25±0.006	834.5±0.003	39.2±0.008	41.23±0.007	<IDL	<IDL
Jarash	<IDL	8.27±0.003	899±0.007	39.1±0.006	40.54±0.0001	<IDL	<IDL
Amman	<IDL	6.60±0.001	1010.5±0.00	26.1±0.001	29.71±0.0001	<IDL	<IDL
Ma'an	<IDL	7.20±0.005	1743±0.006	53.7±0.004	27.80±0.003	<IDL	<IDL
Tafelah	<IDL	7.77±0.006	714.25±0.002	50.4±0.003	42.76±0.001	<IDL	<IDL
IDL	0.005<	0.003<	0.006<	0.002<	0.001<	0.001<	0.002<

* are included in accreditation.

Maric et al. (2006) observed that the oil content in *S. officinalis* was increased in higher altitude than lower. In contrast, the present study showed that the oil content in lower altitude regions of Jarash (520-750 m ab.s.l) and Ma'an (1100-1140 m ab.s.l) was higher than those observed in higher altitudes regions of Tafilah, Ajloun and Amman (2.2-2.8%) (0.87-1.8), respectively. The same results were reported by Haider et al. (2009) and Takaloo et al. (2012) who found that the lowest oil yields were recorded in plants collected from the relatively higher altitude. However, other factors may be induced the essential oil yields in Jarash and Ma'an such as temperature, soil fertility, locality, and the high sunlight radiation level. It was reported that, the aromatic medicinal plants submitted to high level of sunlight radiation yielded higher content of essential oil than others (Letchamo & Gosselin, 1995; Silva et al., 2006). Qasaimeh (2012) showed that the average daily solar radiation for the years (2004-2010) in regions closed to Ma'an was 6215 Wh/m²·d and higher than in Tafilah 5763 Wh/m²·d and Amman 5711 Wh/m²·d. This is indicated by the effect of high sunlight radiation level in Ma'an on the higher yields of oil from *S. officinalis*. Li et al. (2006) who found that the highest oil yield was in sage plants grown at 45% of full sunlight. However, this variation indicates the effect of geographical growth location on the yields of essential oils. Such variations could be explained by the effect of environmental factors related to the predominant climatic and geographical differences on various growth areas (Table 3) (Perry et al., 1999; Chan, 2003; Maksimovic et al.,

2007; Abu-Darwish et al., 2010). This is confirmed by the results of oil content recorded in the worldwide regions of Egypt (1.52-1.65%) (Khalil et al., 2008), Lithuania (0.7-1.4%) (Bernotienė et al., 2007), Bosnia and Herzegovina (0.29%-1.07%) (Maric et al., 2006), Algeria 0.9% (Dob et al., 2007) and Spain 1.4-2.0 (Arraiza et al., 2012).

The concentration of Cu metal ranged from 6.6 in Amman to 9.25 mg/kg in Ajloun. These concentrations were within the average content of Cu in dry plant material which is reported to be 2.0-20 mg/kg by Malencic et al. (2003) indicating that all the investigated growth regions are not contaminated with Cu metal. These results were in agreement with the results of our previous study conducted in the south Shoubak region and showed that Cu concentrations in *S. officinalis* cultivated at 15 and 30 cm inter-row spaces were 7.32 and 7.02 mg/kg, respectively. In contrast, Amr and Đorđević (2000) found that Cu metal concentration in *S. officinalis* grown in Al Dbajbe near Amman-Sahab highway was ranged from 61 to 70 mg/kg and higher than the permissible limits.

Fe is one of the most essential elements for living organism's cells. The results showed a wide variation of Fe concentration in *S. officinalis*. It was ranged from 834.5 to 1743 mg/kg in the northern (Ajloun) and southern (Ma'an) regions of Jordan. However, they were within the concentration limits in dry plant material (1000 mg/kg) (Malencic et al., 2003). These differences in Fe concentrations could be explained by the assumption of Shad et al. (2008) who suggested that high concentration of Fe in plants may be due to the foliar absorption from the surroundings air. This is supported by the results of Fe concentrations in *S. officinalis* cultivated in worldwide regions which revealed a wide variation in the values of Fe concentrations. They were 297.4, 453.77 and 450 mg/kg in Turkey (Başgel & Erdemoğlu, 2006), Serbia (Malencic et al., 2003) and Romania (Ştef et al., 2010), respectively. On the other hand, Yeritsy and Economakis (2002) reported that Fe concentration in leaves could be increased by higher concentration of Fe in the growth nutrient medium indicating that the soils of Ma'an and Amman regions are rich source content of Fe.

Mn concentration in all collected samples of *S. officinalis* from different investigated regions were ranged from 26.1 mg/kg in Amman, the middle region to 53.7 mg/kg in Ma'an and Tafilah, the southern regions of Jordan. The contents of Mn in *S. officinalis* originated in the north regions were 39.2 and 39.1 mg/kg in Ajloun and Jarash, respectively. They were higher than found in the middle and lower than recorded in the south regions of Jordan. However, the concentrations of Mn in *S. officinalis* originated in the different geographical locations of Jordan were higher than the critical threshold for Mn deficiency in plant (<10 ppm), These results were in agreement with previous results obtained from *S. officinalis* grown at 30 cm inter-row spacing and harvested during the vegetative period in the south shoubak region (Abu-darwish et al., 2010).

The results of Zn metal concentration in *S. officinalis* showed a limited variation among the different geographic origin regions. The range varied from 27.80 in Ma'an to 42.76 mg/kg in Tafilah, the southern regions of Jordan. In the north, it was 41.2 mg/kg in Jarash and 40.54 mg/kg in Ajloun while it was 29.71 mg/kg in the middle region. The same findings were observed by Amr and Đorđević (2000) who found that the concentration of Zn in *S. officinalis* originated from middle of Jordan varied from 22 to 29 mg/kg. On the other hand, Malencic et al. (2003) reported that the concentration of Zn in plant may vary between 30-150 mg/kg, but usually it is between 20-50 mg/kg. In general, the low contents of Zn in all studied samples indicated to non polluted soil environment and/or in locations faraway from industrial and heavy traffic activities which were reported the main factors of plant growth soil pollution with heavy metals (Angelova et al., 2005; Blagojević et al., 2009; Stancheva et al., 2009). In conclusion, the results of the present study revealed that *S. officinalis* grown in Jordan is a safe medicinal plant containing sufficient amount of a biological active essential oil that is clearly influenced by metrological and environmental factors. *S. officinalis* originated within investigated southern, middle and northern regions of Jordan are free from hazardous heavy metals such as Cd, Pb. Moreover, the surrounding environments of *S. officinalis* original locations are not polluted with heavy metals. Further studies on the composition of *S. officinalis* essential oil, grown in different Jordan environments are recommended.

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