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Determination of Essential Oils and Heavy Metals Accumulation in *Salvia officinalis* Cultivated in three Intra-raw Spacing in Ash-Shoubak, Jordan

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ABSTRACT

Essential oil yields and heavy metals content were determined in *Salvia officinalis*, cultivated in three intra-raw spacing (15, 30 & 45 cm) in the research farm of Ash-Shoubak University College. Specimens were harvested at the vegetative, beginning of blooming, full-blooming and fruit maturation stages. Essential oil concentration and content of heavy metals in the plant specimens were determined by using hydrodistillation and atomic absorption spectrometry methods, respectively. The yields of essential oil and heavy metal contents were affected by intra-raw spacing and phenological stage. The maximum oil yield was obtained in plant cultivated in 15 cm planting space (2.00) and harvested during vegetative stage, while the minimum oil content was detected in the plants cultivated in the same raw planting space and harvested at maturation stage. Heavy metals contents were variable depending on both intra-raw spacing and phenological stage. Co, Cd, and Pb were not detected. Contents of Ni, Zn, Fe and Cu were increased during the vegetative stage of the plant but still below their toxic level. Results indicated that *S. officinalis* cultivated in 15 cm planting space and harvested at the vegetative stage in Ash-Shoubak is rich in essential oils and free from hazardous heavy metals. © 2011 Friends Science Publishers

Key Words: Aromatic; Pollution; Sage; Soil; Trace elements; Volatile oil

INTRODUCTION

Sage (*Salvia officinalis* L.) is a well-known medicinal and culinary herb that has been used for centuries in the Mediterranean region. The widespread genus *Salvia* has 500 and 900 species (Hedge, 1992). The most popular species of the genus is sage, which is used as spice and flavoring agent in food industry, perfumery and cosmetics. In Jordan, 19 species of the genus *Salvia* are recorded (Al-Eisawi, 1982). Amr and Đorđević (2000) reported that the sage originating from Jordan complied with the standard requirements for the plant species, and can be used as a high quality raw material for the production of phytopreparations. The leaves of *S. officinalis* are used to relive headache, flatulence, toothache, abdominal pain, and common cold or as a sedative agent, wound healing and antidiabetic preparation (Abu-Irmaileh & Afifi, 2003; Otoom *et al.,* 2006; Al-Qura'n, 2009). Several studies have been published indicating the antioxidant and antimicrobial properties of the essential oils extracted from *S. officinalis* (Velickovic *et al.*, 2003a; Velickovic *et al.,* 2003b). Moreover, *S. officinalis* infusions are used as haemostatic, estrogenic, anti-perspiration, anti-neuralgic, spasmolytic, antiseptic, astringent and as hypoglycemic agent (Istudor, 2001).

Heavy metals are detected in many medicinal plants (Başgel & Erdemoğlu, 2005; Razic *et al.,* 2005; Sumontha *et al.,* 2006; El-Rjoob *et al.,* 2008; Abu-Darwish *et al.,* 2009; Massadeh *et al.,* 2009). They can affect the production and yield of certain biological compounds with different roles in living tissues of animals and plants (Malencic *et al.,* 2003; Oktem, 2005; Aziz & Gad, 2007). The content of heavy metals in aromatic medicinal plants may be affected by geochemical characteristics of soil, or location in which the plant is cultivated (Chan, 2003; Abu-Darwish *et al.,* 2009a; Abu-Darwish *et al.,* 2009b). Moreover, rain, atmospheric dust, plant protective agents, and fertilizers could be additive factors of plant contamination with heavy metals (Malencic *et al.,* 2003). On the other hand, the distribution and accumulation of heavy metals among plant species and their organs is found to be selective and depends on the plant species and the individual ability of their parts to accumulate metals

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(Szentmihályi & Csedó, 2002; Prasad & Freitas, 2003; Angelova *et al.,* 2005; Rio-Celestino *et al.,* 2006; El-Rjoob *et al.,* 2008).

The objectives of this study were to determine the essential oil yield and lead (Pb), cadmium (Cd), chromium (Cr), cobald (Co), nickel (Ni), zinc (Zn), iron (Fe), cupper (Cu) and manganese (Mn) contents in *S. officinalis* cultivated in 15, 30 and 45-cm intra-raw spacing in Ash-Shoubak, south of Jordan.

MATERIALS AND METHODS

Location: This study was performed in the research farm of Ash-Shoubak University College, Ash-Shoubak, Jordan (Latitude 30 31N, Longitude 32 35 E). Ash-Shoubak is 1365 m above the sea level. Ambient temperature and seasonal means of rainfall during the studying period in 2007 were (4.11 - 19.9C) and 294.2 mm, respectively. Soil texture in the first 30 cm of the cultivation area can be defined as clay loam, with a pH 7, 6 (Table I).

Plant cultivation: *S. officinalis* seedlings with a well-developed root system were transplanted and cultivated in the experimental area in May of 2007 in 50 cm-rows with 15, 30 and 45 cm intra-row spacing. A drip irrigation system and other field practices had been done without addition of fertilizers. Five plants were harvested from each intra-row spacing at the following phenological stages, vegetative (VEG), beginning of blooming (BB), full-blooming (FB) and fruit maturation (FM) during June, July, August, September 2007, respectively. All the plant samples were dried at about 20C.

Essential oil extraction: A 20 g of the dried and coarsely powdered specimens including leaves, stems, flowers, and fruits (depending on the phenological stage) of *S. officinalis* were steam distilled in triplicate for 2 h using a Clevenger-type distillation apparatus (European Pharmacopoeia, 2005). Yield percentage was calculated as volume of essential oil per 100 g of plant dry matter.

Determination of heavy metals: Heavy metals content were analyzed in selected samples with highest and lowest essential oil contents in 15, 30 and 45 cm intra-raw planting spaces by Atomic Absorption Flame Emission Spectrophotometer, (Model AA-6200 Shimadzu Japan) (Al-Alawi *et al*., 2007). 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 gm of plant sample was wet digested with an ultra-pure nitric acid (HNO_3) (15 mL) in a polyethylene test tube using a heating blocks 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. All reagents used were of analytical grade (Sigma-Aldrich, Switzerland).

Soil samples: Three soil samples, collected in a depth of approximately 0-15 cm from the location of S. *officinalis* growth area, were sieved through 2-mm stainless steel sieve. Samples were dried at 110^{o}C for 24 h to achieve a constant weight. After cooling, the sample was passed through 2 mm sieve and stored in polyethylene bottles for chemical treatment using acid digestion. Acid digestion was performed by placing 0.5 g of soil sample in a beaker and digested with 8 mL mixture of concentrated HCl and HNO_3 with a ratio of (6:2 v/v) for 6 h at 90ºC and 2 mL of concentrated HCl was added. The residue was filtered and diluted to 25 mL with deionized water. The solution was stored in a refrigerator at 4ºC for analysis (El-Rjoob *et al*., 2008).

RESULTS AND DISCUSSION

The essential oil contents of *S. officinalis* samples varied between 0.80% and 2.00% (Table II). Maximum yield was recorded during the VEG stage in inter-raw distance of 15-cm (2.0%) and the minimum was recorded at 45 cm during the FM stage (0.87%). The detected heavy metal contents varied depending upon inter-raw spacing and phenological stage. The contents of Cd, Pb, Cr and Co were not detected in any specimen at all inter-raw planting spaces. Other metal concentrations varied at different inter-raw planting spaces during the VEG and FM stages. Mean levels of Ni ranged from 0.42% to 4.17% at 15 cm inter-raw planting space. Average Fe concentration varied from 524.67% at 45 cm to 935.40% at 15 cm during the VEG and FM stages, respectively. The mean concentrations of Fe, Cu, Zn, Mn, Cd and Pb in all investigated soil samples were lower than the permissible limits (Table I). pH values for soil samples were moderately basic (Table I).

Essential oils: Essential oils extracted from *S. officinalis* were influenced by both, growth stage and inter-raw planting space (Table II). The oil content extracted during VEG and BM stages planted in 15, 30, and 45 cm intra-raw spacing were found to satisfy the requirements of European Pharmacopoeia (EP), while samples collected at FB and FM stages from all studied intra-raw spacing plants were lower than EP. The EP requires an oil yield of 1.0% v/w or above (Anonymous, 2005). The results showed a clear effect of both, intra–raw planting space and phenological stage on the oil yields. The maximum oil yields were 2.00, 1.80 and 1.73% during the VEG stage of *S. officinalis* planted in 15, 30 and 45 cm intra-raw spaces, respectively, which decreased to 0.80, 0.90 and 0.87% during the FM and BB stages, respectively although earlier the highest oil content was detected in four years old *S. officinalis* samples from central Jordan, collected during the blooming phase (2.13%). Nevertheless, the obtained results in both locations were higher than those recorded in other parts of the world (Dob *et al*., 2007; Raal *et al*., 2007; Khalil *et al*., 2008).

In the present study, essential oils extracted during the life cycle stages of all studied inter-raw planting spaces of *S. officinalis* decreased in the order: VEG>BB>FB>FM. Similar trend was observed with an increase in the second

Table I: Means of heavy metals contents (mg/kg) and pH values in 15, 30, and 45 planting space soils

Intra-raw Spacing	Extract		mg/kg						%			
	Ph	EC	Fe	Cu	Zn	Mn	Cd	Pb	Clay	Silky	Sandy	Texture
15 cm	7.8	1.81	6.84	2.85	5.07	25.38	0.12	<0.01	28.0	39.0	33.1	Clay loam
30 cm	7.8	1.40	6.03	2.49	3.43	26.05	0.11	<0.01	27.3	38.1	34.5	Clay loam
45 cm	7.9	1.49	5.13	2.40	2.83	18.09	0.10	<0.01	27.2	38.4	34.4	Clay loam

Table II: The % of oil content in *Saliva officinalis* L. cultivated at 15, 30, and 45 cm planting spaces in Ash-Shoubak region

Inter-raw planting space	Phenological Stage			
	VEG	BB	FB	FM
15 cm	2.00±0.1155	1.8±0.2517	0.84±0.0058	0.80±0.00
30 cm	1.80±0.0058	1.70±0.1155	0.93±0.0058	0.90±0.001
45 cm	1.73±0.1155	1.50±0.1155	0.93±0.002	0.87±0.001

Table III: Concentration of heavy metals (mg/kg) in *Saliva officials* L. cultivated in Ash-Shoubak region depending on phenological stages and intra-raw spacing

Plant Stage	Pb	Cd	Cr	Co	Ni	Zn	Fe	Cu	Mn
VEG /15 cm	Ph	nd	nd	nd	0.42±0.25	116.91±0.0.36	736.17±6.94	7.32±0.81	45.0±0.46
VEG/30 cm	nd	nd	nd	nd	2.78±1.08	95.81±1.19	768.97±5.41	7.02±0.50	51.35±0.42
VEG/45 cm	nd	nd	nd	nd	4.17±1.38	108.85±0.52	524.67±3.35	13.07±0.70	44.63±0.72
FM/15 cm	nd	nd	nd	nd	4.94±1.09	125.71±0.29	935.40±8.84	12.14±0.93	45.00±0.64

nd: not detected

term crop (Mirjalili *et al*., 2006; Zawislak & Dyduch, 2006). Oil concentration is influenced by environmental factors, methods of cultivation, seasonal variations and harvesting time (Qiu *et al*., 2005; Maric *et al*., 2006; Bernotienė *et al*., 2007). Also Qiu *et al.* (2005) reported qualitative and quantitative differences in the oil of *S. officinalis* collected in Shanghai, China at various seasonal periods. The highest yield was observed in three-years-old samples. The increasing effect on essential oil contents during the vegetative stage of plant cycle could also be influenced by light level (Al-Ramamneh, 2009). Light stimulated the production of peltate glanular trichomes, the formation of which is a prerequisite for the accumulation of essential oils in thyme plants. On the other hand, the decreased amount of essential oil from sage samples collected at FB and FM stages may be due to drought caused by high temperature during these periods. A significant reduction in the essential oil content in chamomile when exposed to drought conditions was observed (Razmjoo *et al*., 2008).

Soil pH and electrical conductivity (EC): The pH values of all examined soil samples were moderately basic and were 7.8 in soil samples planted at 15, 30 cm and 7.9 in soil samples planted at 45 cm intra-row spacing. Similar values were recorded earlier for the study area. Nevertheless, they were higher than those reported in Thailand (5.8 to 6.67) (Parkpian, 2003; El-Zuraiqi *et al*., 2004; El-Rjoob *et al*., 2008). EC values ranged from 1.40 to 1.81 in all investigated soil samples which indicated a relative water-soluble salt content in the soil (El-Rjoob *et al*., 2008). The soil in Ash-Shoubak region is similar to those studied in other parts of Jordan and is characterized by its poor fertility due to the fact that the soil is under cultivation for centuries.

Heavy metals in soil: The mean average concentrations of Fe, Cu, Zn, Mn, Cd, and Pb in all soil samples were 6.00, 2.58, 3.78, 23.17, 0.11 and < 0.01, respectively. They were lower than the permissible limits of rare elements in agricultural soils in Canada, USA, France, Germany and United Kingdom. This indicated that the soil growth area of Ash-Shoubak region is clean and not polluted. In a study in Irbid, Jordan, El-Rjoob *et al*. (2008) detected high concentrations of heavy metals in soil samples indicating clearly the effect of condensed traffic on the soil characteristics.

Heavy metals in plant samples: The results of trace element contents are shown in (Table III). Pb was not detected during the VEG and FM pheneological stages of *S. officinalis* planted at 15, 30 and 45 cm inter-raw spaces. The absence of Pb might be mainly due to the cultivation location, distanced from the main road and motor vehicles, the leading factors of plant contamination with Pb (Łozak *et al.*, 2002; Malencic *et al.*, 2003; Prasad & Freitas, 2003; Angelova *et al.*, 2005; El-Rjoob *et al.,* 2008; Massadeh *et al.*, 2009). On the contrary, *S. officinalis* cultivated in two other locations in the central parts of Jordan has been found to be contaminated with Pb. Also *S. officinalis* cultivated in other regions of the world were found to be contaminated with Pb, and exceeded the toxic level (Malencic *et al.,* 2003; Angelova *et al.,* 2005).

Cd is toxic for plants in high concentrations due to its high affinity to –SH groups of enzymes and proteins. The average normal and toxic Cd contents in plant leaves are 0.05-0.20 mg/kg and 3-30 mg/kg, respectively. Cd was not detected in any of the examined specimens of *S. officinalis* collected from Ash-Shoubak region in all inter-raw planting spaces, which was similar to that found in the central regions of the country (Amr & Đorđević, 2000). On the other hand, Cd was detected below the toxic level (3-30 mg/kg) in *S. officinalis* cultivated in other regions of the world. Its value was higher in samples collected near the main roads exposed to high (Malencic *et al.,* 2003; Angelova *et al.,* 2005; El-Rjoob *et al.,* 2008). Likewise, Cr was not detected. As previously reported for central regions of Jordan. High concentrations of Cr were recorded in some other countries such as Turkey and Hungary which differed significantly of the plant species and location of cultivation area (Başgel & Erdemoğlu, 2005).

Co was not detected in the samples of the present study and in the samples from the central parts of the country. This confirms the assumption that Co distribution in plants is entirely species-dependent. The uptake of Co is controlled by different mechanisms in different species. Physical conditions like salinity, temperature, pH of the medium, and presence of other metals in the soil influence the Co uptake and accumulation in medicinal plants.

Low concentrations of Ni are beneficial for plant growth and development, respiration intensity and photosynthesis, as well as for the activity of antioxidant enzymes. The average content of Ni in plant is 0.10 – 5.0 mg/kg, the toxic level ranges from 10 to 100 mg/kg. The lowest content of Ni recorded 0.42 mg/kg at 15 cm inter-raw planting space and harvested during the VEG stage, while the highest was 4.94 mg/kg, during the FM stage at the same inter-raw planting space. Ni content during the VEG stage at 30 and 45 cm inter-raw planting spaces were 2.78 and 4.17 mg/kg, respectively. These results showed that the content of Ni was increased by increasing the inter-raw planting space, indicating to the activity of the root, Ni absorbing system and/or the metabolic activity of the tissue-metal accumulating (Sengar *et al.,* 2008). Low level of Ni might be due to the geographical effect of Ash-Shoubak location, which is windy and wide area, whereas the wind direction affects its concentration in the plant (El-Rjoob *et al.,* 2008).

Zn has an important role in the biosynthesis of enzymes and some proteins. The concentration of Zn in plant may vary between 30-150 mg/kg, but usually it is between 20-50 mg/kg (Malencic *et al.,* 2003). The lowest concentration of Zn (95.81 mg/kg) was found in *S. officinalis* during the VEG stage within 30 cm intra-raw planting space, while the highest was 125.71 mg/kg in plants collected during FM stage in 15 cm inter-raw planting space. On the other hand, Zn content was increased during the life cycle of *S. officinalis* cultivated at 15 cm intra-raw space from 116.91 mg/kg in VEG stage to 125.71 mg/kg during FM stage indicating the Zn accumulation in the leaf tissue of *S. officinalis* (Angelova *et al.,* 2005; Rio-Celestino *et al.,* 2006). Lower concentrations were recorded in *S. officinalis* cultivated in the central region of Jordan (Amr & Đorđević, 2000).

Fe concentration in dry plant material reaches 1000 mg/kg or higher (Malencic *et al.*, 2003). In the present study the concentration of Fe reached its maximum (935.40 mg/kg) at 15 cm inter-raw planting space and harvested during FM stage, while the minimum was 524.67 mg/kg in the samples cultivated within 45 cm inter-raw planting space and harvested during VEG stage. The content of Fe increased proportionately to progressive growth stage of samples planted at 15 cm inter-raw space, where it was increased from 736.17 mg/kg at the VEG stage, to a maximum of 935.40 mg/kg at FM stage. The average Fe values in *S. officinalis*, cultivated in the central parts of the country, ranged between 122 mg/kg to 148 mg/kg in samples collected at BB stage while the maximum level was recorded in samples collected during blooming and FM i.e., 184 and 182 mg/kg, respectively. The high content of Fe in *S. officinalis* could be attributed to the absence of Co, since high levels of Co induce Fe deficiency in plants and suppress uptake of Cd by roots. These results seem to indicate that both soils and plants in Ash-Shoubak south region are well supplied with this essential microelement compared to the central regions of Jordan and other geographical locations reported in the literature (Malencic *et al.,* 2003; Başgel & Erdemoğlu, 2005).

Cu is an essential microelement for plants. The average content of Cu in dry plant material is reported to be 2.0-20 mg/kg (Malencic *et al.,* 2003). It affects respiration, metabolism of carbohydrates, lipids and proteins. In the present study the concentrations of Cu in all samples of *S. officinalis* were within the normal range and lower than those recorded in the central region of Jordan and ranged between 7.02 and 13.07 mg/kg during the VEG stage of *S. officinalis* planted at 30 and 45 cm inter-raw planting spaces, respectively. On the other hand, the contents of Cu in *S. officinalis* grown in Serbia, Yugoslavia, Turkey and Bulgaria were 25.1, 26, 5.5 and 35.8, respectively (Malencic *et al.,* 2003; Angelova *et al.,* 2005; El-Rjoob *et al.*, 2008). These findings could be due to the effect of anthropogenic activities and heavy traffic observed in these regions that may accumulate Cu in the soil (Razic *et al.,* 2005; El-Rjoob *et al.,* 2008). Cu availability for plant uptake is dependent upon number of plant factors such as root intrusion, water and ion fluxes and their relationship to the kinetics of metal solubilization in soils; biological parameters, including kinetic of membrane transport, ion interactions and metabolic fate of absorbed ions.

The contents of Mn in all samples of *S. officinalis* were similar. The highest content was 51.35 mg/kg in samples cultivated at 30 cm inter-raw spacing and harvested during the VEG stage, while the lowest was 44.63 mg/kg at 45 cm inter-raw space. All findings were lower than those detected in the central regions of Jordan, which was ranged from 92 to 108 mg/kg. The recorded data were higher than those recorded in Serbia and Turkey, where the content of Mn was 39.25 and 32.6 mg/kg, respectively (Malencic *et al.,* 2003; Başgel & Erdemoğlu, 2005). These variations could be due to the differences in plants uptake, which can be explained by differences in the ability of plants to bring about the dissolution of oxidized manganese.

CONCLUSION

The yields of essential oil and of heavy metals content in *S. officinalis* are affected by phenological stages and intra-raw planting spaces. *S. officinalis* L. cultivated in 15 cm planting space and harvested during the VEG stage in Ash-Shoubak is rich in essential oils content and free from hazardous heavy metals.

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