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HEAVY METALS CONTENT AND ESSENTIAL OIL YIELD OF *Juniperus phoenicea L.* IN DIFFERENT ORIGINS IN JORDAN

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Abstract

The present study was conducted to determine the heavy metals (Fe, Mn, Zn, Cu, Pb, and Cd) and essential oils content in the berries of *Juniperus phoenicea L.* grown in two different geographic origins; Dana conservation and Al-Hisha forest in Jordan. Atomic absorption and Hydrodistillation methods were used to determine heavy metals concentrations and oil contents in plant samples of *J. phoenicea L.*, respectively. Results showed that, the oil yielded from *J. phoenicea* L. berries in Al-Hisha was higher than that grown in Dana area (3.7 and 0.9%). Heavy metals contents were in variable concentrations in the order: Fe > Cu > Zn > Mn > Pb and Cd .Cu concentration in *J. phoenicea L.* berries varied from 31.51ppm in Dana to 71.86 ppm in Al-Hisha. It was higher than the maximum normal limits in plants (2.0-20 ppm). The concentrations of Fe, Zn, and Mn were very low and did not exceed their typical amount in non polluted plant. Toxic heavy metals Pb and Cd were not detected in all plant samples. The results indicated that, *J. phoenicea* L. can be used as an indicator of copper bioavailability from surrounding environment. The Berries of *J. phoenicea L.* grown in the south area of Jordan were rich in essential oils content but it was affected by geographic growth origin.

Key words: essential oil, heavy metals, Jordan, *Juniperus phoenicea L.*

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1. Introduction

Juniperus phoenicea L. is an important aromatic medicinal plant belonging to the Cupressaceous family. It is distributed in mountain regions and characterizes the woody vegetation in Tafileh and Shoubak woodlands in the south part of Jordan (Al-Qura'n, 2008, 2009). *J. phoenicea L.* is endemic medicinal plant to the northern lands bordering the Mediterranean Sea from Portugal to Israel as well as Canary Islands (Adams et al., 1996). Essential oils of berries and leaves of *J. phoenicea L.* exhibited certain biological functions that could act as antioxidant (Ennajar et al., 2009), antimicrobial (Angioni et al., 2003; Derwich et al., 2010) and antifungicidal agents (Cosentino et al., 2003). Leaves and berries are used in Jordan for the treatment of various diseases such as diabetes (Hamdan and Afifi, 2004), rheumatism (Al-Qura'n, 2009), and as antidiarrheal agent (Qnais et al., 2005).

Certain aromatic medicinal plants have shown to uptake and accumulate toxic heavy metals from polluted areas, and could be used as biomonitors or accumulators of pollutants (Abu-Arab and Abou Donia, 2000; Achak et al., 2009; Başgel and Erdemoğlu, 2005; Diaconu et al., 2009; Djingova et al., 2003; Hiçsönmez et al., 2009; Kurteva, 2006;Sagiroglu et al., 2005). However, limited studies of heavy metals contents were conducted in Jordan (Abu-Darwish, 2009; Abu-Darwish et al., 2009; Abu-Darwish et al., 2010; Al-Shayeb et al., 1995; Amr and Đorđević, 2000; El-Rjoob et al.,

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2008; Jaradat and Momani, 1999). Also, few studies have focused on heavy metals uptake by *J. phoenicea L*. Therefore, the present study was designed to evaluate *J. phoenicea L.* for the heavy metals accumulation (Fe, Mn, Zn, Cu, Pb, Cd) and to find out the effect of geographic growth origin on the yields of essential oil in the berries of *J. phoenicea L.*

2. Material and methods

2.1. Soil samples

Three soil samples were collected from selected plant samples origin in both regions at two depths: 0-10 cm and 10-20 cm simultaneously. pH was determined in water with a 1:2.5 soil to solution ratio, and electrical conductivity was measured (Page et al., 1982).

2.2. Determination of Heavy metals contents

2.2.1. Heavy metals in plant samples

Heavy metals contents in plant samples of *J. phoenicea L*. were determined by atomic absorption method (Al-Alawi and Mandiwana, 2007) using Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan). The unwashed crushed and mild plant samples of *J. phoenicea L*. berries were oven dried at 70°C for 24 h until the dry weight was constant. Dried plant samples were treated with pure nitric acid and heated at 120^{o}C in heating block digestion unit. The final solution was filtered through a 45-µm filter paper and diluted with pure water.

2.2.2. Heavy metals in soil samples

Soil samples were dried at 110°C for 24h then cooled to achieve constant weights. 0.5 g of dried soil samples were digested with 8 ml of concentrated HCl and HNO3 mixture (6:2 v/v) for 6 h at 90°C following by addition of 2 ml of concentrated HClO4. The mixtures were filtered and diluted to 25.0 mL with deionized water. Analysis of the filtered mixture was conducted by Atomic Absorption method using Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) (Tuzen, 2003).

2.3. Medicinal plant samples

Three samples of *J. phoenicea* L. ripe berries were collected randomly, from two different habitats in the south part of Jordan; Dana conservation area (Tafilah province) and Al-Hisha (Shoubak province) which have limited industrial activities and low traffic density. The ripe berries were collected in summer 2010 and air dried in shadow.

A voucher specimen was deposited at the Herbarium of Ash-Shoubak University College. The dried samples of *J. phoenicea* L. berries were separately crushed into small pieces and sieved through (0.5 mm) mesh sieve.

2.4. Essential oil extraction

50gr of crushed dried plant materials were separately submitted to steam water distillation using a Clevenger-type apparatus (European Pharmacopoeia, 2005). Distillation time was 3 h at a rate of 2-3 drops min^{-1}. The values reported are the mean of at least three distillations and three replications for each specimen.

3. Results and discussion

3.1. Essential oil yield

The investigated plant in our present study was collected randomly from the most two origin regions for it (Dana & Al-Heisheh). The collection procedure was made randomly in the ripe growth stage in the same period considering that the investigated plant is grown (wildly) and its berries are used in Jordanian folk medicine in the ripe form, so that, our study was focused on the essential oil yield and heavy metals contents mainly during the ripe stage of berries.

The yields of essential oil of *J. phoenicea L.* berries are listed in Table 1). Results showed that, essential oil content in berries of *J. phoenicea L.* grown in Al-Hisha was higher than that grown in Dana conservation area (3.7% and 0.9 %, respectively). Such differences in the yield could be indicated by the effect of geographic and different climatic characteristics in the two studied growth locations as shown in Table 1. Razmjoo et al. (2008) found that drought could significantly reduce the essential oil content in plants. Furthermore, the difference in the altitudes in Al-Hisha (1365 m) and Dana conservation areas (1260 m) may be an additional factor affecting the contents of essential oils. This is confirmed by the results of Martz et al. (2009) who observed that the concentration of phenolic and terpenoid compounds in *J. communis* needles were clearly increased with latitude and altitude. On the other hand, Figueiredo et al. (2006) reported that some physiological variations, environmental conditions, geographic variations, and genetic factors could affect the contents of essential oil in plants (Figueiredo et al., 2006). Moreover, some heavy metals were reported to affect the essential oil yields of aromatic medicinal plants (Stancheva et al., 2009; Zheljazkov et al., 2008). In the present study, the low concentration of Fe in the growth soil samples in Al-Hisha (1.81-3.45ppm) may stimulate higher yield of essential oil compared to those of Dana conservation growth area (Table 2). Yeritsyan and Economakis (2002) found that the high concentration of Fe in growth culture of the plant can reduce the essential oil yield. In the present study, the yield of essential oil extracted from berries of *J. phoenicea* L. and grown in Al-Hisha region was higher than the minimal requirements (1%) recorded in European Pharmacopoeia (2005).

Also, it was higher than yields obtained from various parts of J. spp. grown in other regions of the world (Achak et al., 2009; Adams et al., 1996; Angioni et al., 2003; Derwich et al., 2010; El Sawi et al., 2007).

3.2. Soil texture, pH and electrical conductivity

The characteristics of soil samples such as pH and electrical conductivity (EC) are shown in Table (2). The sieve and hydrometer analysis of soil samples showed that the grain size particle was composed of 67.6-74.4% sand, 3.3-3.4% clay and 22.3-29.0% silt in Dana region which represented a sandy loam texture.

The soil samples from Al-Hisha region contained 31.1-31.4% sand, 28.6-30.9% clay and 38.0-39.9% slit which represented a clay loam. These results were in agreement with previous studies (Al-khashman and Shawabkeh, 2006; El-Zuraiqi et al., 2004). pH results for all examined soil samples were moderately basic (7.7-7.9 Vs 7.7-7.8) in Dana and Al-Hisha regions. The EC values in the two locations were different. They ranged from 1.06 in the upper layer to 1.62 mS /cm in the lower soil layer in Dana and increased from 1.76 mS/cm to 3.70 mS/cm in Al-Hisha region.

These results were similar to results obtained by EL-Zuraiqi et al. (2004). They found that pH and EC values were 7.6-7.9 and 1.4-2.5 mS /cm, respectively, in Ash-Shoubak. In contrast, Al-khashman and Shawabkeh (2006) found that pH values of soil samples around the cement factory in Qadissiya area and close to Dana conservation area were 7.23 - 8.15 and the average values of EC ranged between 0.470 mS/cm in upper soil surface and 0.443 mS/cm in the lower surface.

3.3. Heavy metals in the soil

Heavy metals content in soil samples are presented in Table (2). In general, there were low concentrations of metals in both soil samples. The upper surface of soil samples in Dana location contained relatively higher concentrations of heavy metals than lower surface. They were 9.25, 10.01, 10.01, 0.86, and 0.23 ppm for Fe, Mn, Zn, Cu and Cd in the upper surface (0-10cm) and were 4.44, 2.84, 0.60, 0.46, 0.07ppm, in the lower surface (10-20cm), respectively. These differences could be explained by the effect of physical properties of soil and its alkaline pH values (Al-khashman and Shawabkeh 2006; Vecera et al., 1999).

The contents of Fe, Zn, Mn and Cd in the upper surface of the soil of Dana region were higher than recorded in the upper surface of Al-Hisha region. However, heavy metals contents in both locations were lower than reported in neighboring soil samples or in other locations in Jordan (Al-khashman and Shawabkeh, 2006; El-Rjoob et al., 2008). These results indicated that the soil of Al-Hisha and Dana regions were not polluted by waste of vehicles since its location is far away from heavy traffic compared to a previous study carried out in a big and condensed traffic Irbid city in Jordan, which showed high concentrations of heavy metals in soil samples (El-Rojoob et al., 2008).

3.4. Heavy metals in plant samples

The concentrations of Fe, Mn, Zn, Cu, Pb and Cd in the studied samples of *J. phoenicea L.* berries are summarized in Table 3. As evident from this table, there were differences in the values of tested heavy metals in *J. phoenicea L.* collected from Dana and Al-Hisha regions.

Table 1. Locality, climate conditions, altitudes, latitudes and essential oil yield of *J. phoenicea L.* grown in two different geographical locations of Jordan

Growth Location	*Altitude (m)*	*Ambient Temp. (Min-Max)*	*Mean of Rainfall (mm)*	*Relative Humidity (%)*	*Latitude (degrees)*	*Essential Oil %*	
						Range (n=3)	*Average* (n=3)
Dana	1260	11.8-23.4	237.6	23.30	30.4030° N	0.86-0.93	0.90 ±0.04
Al-Hisha	1365	4.11-19.9	294.2	24.80	30.3100° N	3.23-3.97	3.70 ±0.41

Table 2. Descriptive parameters and concentration of heavy metals (in ppm) in studied soil samples based on the dry mass (mean ±SD)

Location	*Depth/ cm*	*Extract*		*Ppm*						*%*			*Texture*
		PH	*EC*	*Fe*	*Cu*	*Zn*	*Mn*	*Cd*	*Pb*	*Clay*	*Sand*	*Silt*	
Dana	10	7.7	1.62	9.25 ±0.013	0.86 ±0.004	10.01 ±0.003	10.01 ±0.001	0.23 ±0.083	<0.01	3.4	67.6	29.0	Sandy Loam
Dana	20	7.9	1.06	4.44 ±0.022	0.46 ±0.003	0.60 ±0.006	2.84 ±0.053	0.07 ±0.058	<0.01	3.3	74.4	22.3	Sandy Loam
Al-Hisha	10	7.7	1.76	1.81 ±0.033	0.93 ±0.016	0.34 ±0.003	4.53 ±0.026	0.03 ±0.005	<0.01	28.6	31.4	39.9	Clay Loam
Al-Hisha	20	7.8	3.70	3.45 ±0.005	1.11 ±0.006	0.32 ±0.006	4.19 ±0.076	0.05 ±0.015	<0.01	30.9	31.1	38.0	Clay Loam

Table 3. Heavy metals contents (ppm) in berries of *J. phoenicea L.* grown in Dana reservation and in Al-Hisha (mean ±SD)

	Metal in (ppm).					
Sample	***Fe***	***Mn***	***Zn***	***Cu***	***Pb***	***Cd***
Dana	168.07±0.013	11.29±0.003	40.71±0.003	31.51±0.013	<IDL	<IDL
Al-Hisha	190.11±0.014	6.60±0.00	13.72±0.106	71.86±0.102	<IDL	<IDL

IDL: Instachange Displays Limited

In general, the concentration order of tested heavy metals in plant samples was Fe > Cu > Zn > Mn except for Cu and Zn concentrations in Dana location.

Levels of Pb and Cd were not detected because they were less than the Instachange Displays Limited (IDL). Such variations may be originated from various causes related to soil-to-plant transfer pattern of the heavy metals (Maharia et al., 2010; Vecera et al., 1999). Soil-to-plant transfer process depends mainly on the surrounding environment in which the studied plant were grown (Abu Darwish et al., 2009; Abu–Darwish et al., 2010; Amr and Dordevic, 2000; Barthwal et al., 2008; Diaconu et al., 2009).

The highly toxic heavy metals lead (Pb) and cadmium (Cd) (Shad et al., 2008) were not detected in any of the examined specimens of *J. phoenicea L.* The same findings were observed for *Thymus vulgaris*, *Thymus serpyllum* and *Salvia officinalis L.* in the same regions (Abu-darwish, 2009; Abu-darwish et al., 2009; Abu- Darwish et al., 2010). Results in Table (3) showed that the Fe concentration in *J. phoenicea L.* berries grown in Al-Hisha and in Dana reservation was 190.11 and 168.07 ppm, respectively, it was lower than the typical limits (1000 ppm) reported by Malencic et al. (2003). Fe content in *J. phoenicea L.* grown in Al-Hisha region was higher than recorded in Dana reservation in spite of its lower concentrations in surrounding soil depths as compared to Dana Reservation (Table 2). These differences 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. The concentration of Mn in *J. phoenicea L.* berries ranged from 6.60 ppm in Al-Hisha and 11.2 ppm in Dana, however, this range did not exceed the permissible limit (200 ppm) (Haider et al., 2004). Zn is a highly mobile metal in the soil and can be easily taken up by plants and thus can accumulate in the biomass up to high concentrations (El-Rjoob et al., 2008; Vysloužilová et al., 2003).The content of Zn in berries of *J. phoenicea L.* grown in Dana (40.71ppm) was higher than in berries of *J. phoenicea L.* grown in Al-Hisha (13.72 ppm). This variation of Zn contents may be due to differences in the Zn concentration in the two growth soils: higher content of Zn in the upper and lower soil layers in Dana reservation than in its corresponding soil layers in Al-Hisha (Table 2). However, it was within normal safe limits (100 ppm) (Haider et al., 2004).

Cu is an essential microelement for plants. The average content of Cu in dry plant material is reported to be 2.0-20 ppm (Malencic et al., 2003). In the present study the average content of Cu in berries of *J. phoenicea L.* was 31.51ppm and 71.86 ppm in Dana and Al-Hisha regions, respectively. These results revealed that Cu contents in both studied growth regions were strongly higher than the maximum normal limit of safe Cu in plants (Haider et al., 2004). However, Cu contents in soil samples were relatively low in both locations, 0.46-0.86 and 0.93-1.11ppm in Dana and Al-Hisha, respectively, indicating that the berries of *J. phoenicea L.* could accumulate higher amount of Cu as compared to Cu levels in the soil where they grown.

4. Conclusions

The results of the present study indicated that, *J. phoenicea* L. can be used as an indicator of copper bioavailability from surrounding environment. The essential oil yields and heavy metals content in berries of *J. phoenicea L.* were affected by different geographic growth environment. Berries of *J. phoenicea L.* grown in the south area of Jordan were rich of essential oil content. On the other hand, further studies for essential oils quality are recommended.

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