

Original Research Article



Heavy Metals in Selected Medicinal Plants Originated in Dana Biosphere Reserve, Jordan

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Abstract

A study was conducted to determine the concentration of certain essential and non-essential heavy metals (Fe, Mn, Zn, Cu, Pb and Cd) in selected medicinal plants i.e. Anemone coronaria L., Globularia arabica L., Iris petrana L., Salvia ceratophyla L., Salvia lanigera L., and Salvia multicaulis vahl grown in their different geographic natural environment and originated in Dana Biosphere Reserve in Jordan, The concentrations of heavy metals were estimated by atomic absorption spectrometry method. The results showed that Iron (Fe) metal recorded the highest concentration among all studied plant samples. Its content ranged from 128.27 in A. coronaria to 979.64 ppm in S.lanigera. The concentration order of tested heavy metals in all studied plant samples was Fe> Zn > Mn > Cu > Pb. The most toxic heavy metals, Pb and Cd were detected in all studied samples. Pb in all studied samples except for S. ceratophylla was lower than limits (10 ppm) reported by WHO. Concentrations of Cd in all studied plants were higher than permissible levels reported by FAO/WHO, for edible plants (0.21 ppm). Concentrations of other heavy metals particularly manganese (Mn), zinc (Zn) and Cu were also detected and were exceeded limits reported by FAO/WHO. In conclusion, all of the investigated plant samples originated and grown in Dana Biosphere Reserve were rich in iron Fe metal and contained higher concentrations of a toxic Cd heavy metal than reported by FAO/WHO. However, this study revealed that medicinal plants have a selective ability to absorb and accumulate toxic heavy metals from surrounding atmosphere. Keywords : Dana Biosphere Reserve, Heavy metals, Medicinal plants.

Introduction

Jordan is relatively a small country situated at the junction of the Levantine and Arabian areas of the Middle East. Jordan retains a rich diversity of animal and plant life that varies between Jordan valley, the mountain heights plateau and Badia desert region. Around 2500 plant species were recorded in Jordan flora. There are 485 species belonging to 99 different families are categorized as medicinal plants [1]. Dana Biosphere Reserve is the largest nature reserve in Jordan, it was established in 1989. It is the only reserve in Jordan that includes the four different bio-geographical zones of the country; Mediterranean, Irano-Turanian, Saharo Arabian and Sudanian penetration. More than 800 plant species can be found within the Dana reserve [2], many of them are used in local folk medicine for treatments of various diseases. Anemone coronaria, Globularia Arabica, Iris petrana L., Salvia ceratophylla, Salvia lanigera, Salvia multicaulis vahl, are among the medicinal plants that grow in Dana, where I. petrana is considered as endemic species to the area while G. Arabica is medicinal rare [3]. Several studies were conducted and reported in Jordan to unveil their medicinal importance (Table.1).

People in Jordan, as well as about 75-80% of the world's population mainly in developing countries, still relies on plantbased medicines for their primary health care [4]. On the other hand, the average annual volume of medicinal and aromatic plants utilized in EU countries has been increased by 21 percent since 1992 [5]. Americans have spent \$26.7 billion on alternative therapies including medicinal plants in 2009 [6]. The safety and quality of herbal medicines have become increasingly important concerns for public and international health authorities. Medicinal plants contain primary constituents such as macro and microelements, which were demonstrated to play an important biological role in human, animal and plant health and also can be partially responsible for the medicinal and nutritional properties of medicinal plants, as well as their toxic ones [7]. Elements that are required in amount less than 100 mg/day are known as micro or trace metals. Some micro metals are essential nutrients (zinc, iron, copper, and chromium), and can become toxic at high concentrations, while others (lead, mercury, arsenic and cadmium) have no known beneficial properties and are toxic even at extremely low concentration [7]. They have the ability to bio-accumulate and destroy functions of vital organs and glands in the human body such as liver, brain, and kidney [7]. They can affect the production and yield of certain biological compounds with different roles in living tissues of animals and plants [8]. Heavy metal toxicity in the food chain, especially in plants, represents one of the major environmental and health problems of the contemporary society [9].

Table 1: Studied Plants of Dana Biosphere Reserve; Major Reported Folk Therapeutic Activities, their Phytochemical Constituents, and Latest Common Pharmacological Findings.

Plant name	name Therapeutic activities		Reported Phytochemicl constituents	Reported pharmacological activities		
Anemone coronaria L.	Flowers	Ranuncul- aceae	Shake- ikanuman	Antirheumatic,Antis pasmodic, for cough, pulmonary disease, insomnia [20].	Triterpene and glycosides [21].	Antioxidant, Cytotoxic activity [22].
<i>Globularia arabica</i> L.	Aerial parts	Globularia- ceae	Zreaqa	Anticancer [23].	None	Antimicrobial activity [24].
Iris petrana L.	Flowers	Iridaceae	Sawsan	Expectorant, for teething infants [25]	Iso flavonoids such as iridin, iriline A, irisoneB [26]. Nigricin ,nigricanin, iroline. Xanthon such as mangiferin [6].	Anti -inflammatory, antioxidant activity, antiseptic, pain relief, antibacterial and antifungal [26].
Salvia ceratophylla L.	Aerial parts	Lamiaceae	Miramia	Treat microbial infections, cancer, malaria, inflammation and to disinfect homes after sickness [28].	Essential oil ;γ muurolene and -pinene are the major compounds [28].	Antioxidant activity [28].
<i>Salvia</i> <i>lanigera</i> Desf.	Aerial parts	Lamiaceae	Miramia	Aromatic tea for a variety of abdominal troubles [29].	Essential oil [29].	Cytotoxic activity [30]. Antimicrobial and Antioxidant activity [31].
Salvia multicaulis vahl.	Aerial parts	Lamiaceae	Miramia	Herbal tea and as antiseptic and wound healing purposes [32].	Essential oil predominated 1,8-cineole, camphor, alpha-pinene [33].	Antituberculous activity, Antiviral and Cytotoxicity [34].

Medicinal plants can be easily contaminated with heavy metals from the environment including soil, water, or air and also during industrial processing and packaging [10]. The polluted rainfall, atmospheric dust, plant protective agents, and fertilizers can be additional sources of medicinal plants contamination by heavy metal [11]. The level of essential elements in plants is conditional, the content being affected by geochemical characteristics of the soil and by the ability of plants to selectively accumulate some of these elements [12].

In comparison with other pharmaceutical ingredients and drugs which are regulated by Current Good Manufacturing Practices (CGMP), Good Laboratory Practices (GLP) and other various compendia such as United States Pharmacopeia (USP) and European Pharmacopeia (EP), herbal products are not subject to the same regulations [13]. The one of the most important criteria

for quality assurance of the raw material and finished herbal product is less toxicity and minimum heavy metals [14], even in the most developing countries, the medicinal herbs and their final products are poorly regulated, nor controlled [15]. Thus, detection of heavy metals in medicinal plants and their final products still one of the criteria that determine the safety use of the plant material in the production of traditional medicines and herbal infusions [16].

To the best our knowledge, there were no studies reported for the heavy metals content in *A. coronaria, G. Arabica, I. petrana L., S. ceratophylla, S. lanigera,* and *S. multicaulis vahl* in Jordan. the control of heavy metals in medicinal plants, quality, efficacy, and assurance of the safety of medicinal plants and herbal products especially in the terms of heavy metals should be assured, and has now become a key issue in industrialized and in developing countries. In continuous to our previous work on the heavy metals

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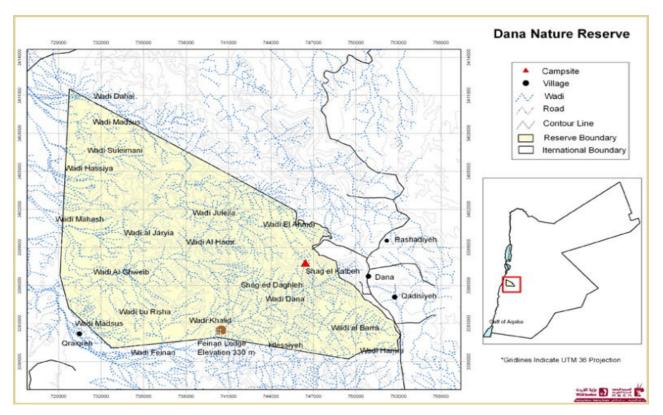
investigation on the medicinal plants of Jordan [17, 18, 19]. Therefore, the aim of this study was to estimate the heavy metals content in the most important selected medicinally plants and originated in Dana Biosphere Reserve in Jordan.

Material and methods

Plant Materials

In order to collect previously reported medicinally important plants, a field trip was arranged in Spring of 2013 throughout Dana Biosphere Reserve (DBR) (Figure 1). Three samples of flowers of *A. coronaria* and *I. petrana* and the areal parts of *G.arabica*, *S.ceratophylla, S. lanigera* and *S. multicaulis* were collected randomly, from different habitats in DBR. Identification and nomenclature of the species was achieved by Dr.Khaled Abulaila from Plant Genetic Resources/Directorate of Biodiversity, National Center for Agricultural Research and Extension (NCARE), Balqa'a-Jordan. Voucher specimens were deposited at the Herbarium of Ash-Shoubak University College.





Determination of Heavy Metals Content in Plant Samples

The content of Pb, Cd, Zn, Cu, Fe, and Mn in particular plants samples were analyzed using Atomic Absorption Flame Emission Spectrophotometer Varian Spectro AA.200 [35]. 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 (HNO3 (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- μ m filter paper and diluted to the mark with ultra-pure water. Ultra-pure water was used for all dilutions and sample preparation. The values reported are the mean of at least three measurements and three replications for each specimen. Analytical results have evaluated by statistical analysis system. The standard error values of the means were calculated to compare the site categories.



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

Table 2.Operating Parameters for Working Elements

The concentrations of Fe, Mn, Zn, Cu, Pb and Cd in the studied samples of *A. coronaria, G. Arabica, I. petrana, S. ceratophylla, S. lanigera, S. multicaulis* are summarized in table (3). As evident from this table, there were differences in the values of tested heavy metals in all investigated plant samples. the concentration order of tested heavy metals in studied plant samples was Fe> Zn > Mn > Cu > Pb > Cd. In general, the concentration order of tested heavy metals was in agreement with the results obtained from Juniperus phoenicea L. grown in Dana, and were in the contrary for Pb and Cd which were not detectable [36]. However, this variation of heavy metal levels may be due to the distribution and accumulation ability of heavy metals among plant species and their organs which are selective and depends on the plant species and the individual ability of their parts to accumulate metals [37,38].

Results and Discussion

Table 3: Heavy Metal Concentrations (ppm)	in Studied Medicinal Plants from Dana Biosphere Reserve
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Studied Sample	Fe	Cu	Mn	Zn	Pb	Cd
Anemone coronaria L.	128.27	16.23	21.67	23.92	5.49	1.7
	±0.100	±0.008	±0.008	±0.005	±0.001	±0.001
<i>Globularia arabica</i> L.	382.04	6.88	27.23	18.45	3.99	1.65
	±0.910	±0.001	±0.008	±0.006	±0.001	±0.001
Iris petrana L.	892.61	8.29	38.66	45.52	6.49	1.45
	±0.730	±0.003	±0.100	±0.021	±0.008	±0.001
Salvia ceratophylla L.	453.68	8.24	41.87	32.41	10.48	1.4
	±0.100	±0.003	±0.080	±0.030	±0.003	±0.002
<i>Salvia lanigera</i> Desf.	979.64	7.35	40.81	47.87	1.49	1.54
	±0.710	±0.001	±0.080	±0.080	±0.002	±0.000
Salvia multicaulis vahl.	605.22	4.88	39.42	27.21	1.00	1.54
	±0.200	±0.002	±0.060	±0.007	±0.002	±0.003
Average Concentration (ppm)	573.58	8.65	34.94	32.56	4.82	1.55

Iron (Fe) recorded the highest average concentration (573.58 ppm) among all studied samples. Its content ranged between 128.27 in *A. coronaria* and 979.64 ppm in *S.lanigera*. These high levels of Fe contents except for A. coronaria were higher than founded in the berries of J. phoenicea grown in the same region and higher than that founded in *Thymus serpyllum* [17] but lower or resemble to thus obtained in *Salvia officinalis* originated in other regions of Jordan [18]. This is may be due to the fact that the level of essential elements in plants is conditional, and the plant selective ability to accumulate metals, and geochemical differences of soil

characteristics [12]. However, the content of Fe in all studied plants was lower than typical limits (1000 ppm) [39] and higher than permissible limit stated by FAO/WHO [40]. in edible plants (20 ppm). However, the WHO did not establish the limits of Fe in medicinal plants [16].

Zn content in all studied samples was higher than the permissible limit (27.4 ppm) stated by [40] for edible plants The lowest content of Zn was recorded in G. Arabica, while the highest was recorded in *S.lanigera* (47.87ppm) followed by *I. petrana* (45.52 ppm). Furthermore, the obtained results revealed that there are variations



in the content of Zn among Salvia species, where the lowest Zn concentration was found in S. multicaulis (27.21 ppm) and S. ceratophylla (32.41 ppm). However, the concentration of Zn in all studied samples were not exceeded the usual amount (20-50 mg/kg) that can be found in plant [41] but in S.lanigera, I. petrana and S. ceratophylla was exceeded the permissible limit (27.4 ppm) reported by FAO/WHO [40]. However, the dietary limit of Zn is 100 ppm. It is an essential trace element form plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing [42,43].

Mn concentration was ranged between 21.67 ppm in the flowers of A. coronaria and 41.87 ppm in aerial parts of S. ceratophylla. The concentrations of Mn in all studied samples were higher than the critical threshold for Mn deficiency in plant (<10 ppm) and exceeded the permissible limit in edible plants reported by FAO/WHO (2 ppm), but not exceeded the limits (200 ppm) indicated by [41]. However, these results were in contrary with our previous results obtained from the ripe fruits of J.phoenicea and grown in Dana Biosphere Reserve, where the content of Mn was 11.29 ppm [36]. Furthermore the content of Mn in all studied Salvia species was lower than previously detected in *Salvia officinalis* (50.4 ppm) and originated in the same province [19].

The lowest concentration of Cu was found in *S.multicaulis* (4.88 ppm), while maximum was recorded in *A. coronaria*. The contents of Cu in Dana Biosphere Reserve were lower than that recorded in ripe berries of *J. phoenicea* grown in the same location, where Cu concentration was 31.51ppm. [36]. However, the contents of Cu in all studied samples were within the average content of Cu (2.0-20 ppm) in dry plant material, but exceeded permissible limit reported by FAO/WHO [40] for edible plants (3.00 ppm).

The highest content of Pb (10.48) was recorded in S. ceratophylla, while the lowest was recorded in S.multicaulis (1.00 ppm). The contents of Pb in all studied samples except for S. ceratophylla. were below the limits (10 ppm) established by WHO [16], but were higher than the level (0.43 ppm) estimated by FAO /WHO [40]. On the contrary, the level of Pb in ripe berries of J.phoenicea collected from Dana Biosphere Reserve was not detectable [36]. However, the content of Pb in all studied samples was lower than that defined in some of other medicinal plants originated in Jordan. The level of Pb in Thymus vulgaris originated in different regions of Jordan was varied between 22.38 to 37.82 ppm [18]. The highest contents of Pb were detected in the vegetative organs of Rosmarinus officinalis, and ranged 10.6-86.5 ppm, [44], while the high content of Pb in Salvia officinalis, collected from various regions of Jordan, rangeed from 17 to 32 ppm [19]. Pb content in seventy-nine dry medicinal plants collected from herbalist shops in Jordan ranged from 3.0 µg in Nigella sativa up to 33.4 µg in Inula viscose [45]. The low content of Pb in our studied samples could be due to the studied plants were collected from Dana Biosphere Reserve which were far away from busy traffic sites, where the

contamination of plants by high concentration of Pb was mainly affected by motor-vehicle exhausts [11]. The contamination of *Malva parviflora* by Pb and other toxic metals were increased by decreasing the distance from the main roads [43].

Cd metal was detected in all tested plant samples. It was ranged from 1.4 ppm in S. ceratophylla up to 1.7 ppm in A. coronaria. The obtained results of Cd concentrations in all studied plants were higher than permissible levels estimated by FAO/WHO for edible plants (0.21 ppm) [40] and permissible levels estimated by WHO (0.3 ppm) [16]. However, Cd did not exceed the toxic level (3-30 ppm) in the plant. In similar study on J. phoenicea originated in the same location Cd was found in the soil samples, while in plant part was not detected [36]. These results were confirmed by [11] who found that relatively high concentrations of cadmium (5.00±2.19 ppm) in the soil samples of the investigated area in the south of Jordan. They explained that, the anthropogenic sources such as cement industry, agriculture activities and traffic emissions seemed to be the responsible source of pollution for metals. On the other hand, these different detectable levels of Cd indicated that the investigated plants have a selective ability to absorb and accumulate Cd from surrounding atmosphere. The accumulation and distribution of heavy metals in plants depend on plant species, element species, chemical and bioavailability, pH, cation exchange capacity, dissolved oxygen and temperature [46].

Conclusion

In conclusion, all of the investigated plant samples originated and grown in Dana Biosphere Reserve were rich in iron Fe metal and containing higher concentrations of a toxic Cd heavy metal than reported by FAO/WHO. However, this study revealed that medicinal plants have a selective ability to absorb and accumulate toxic heavy metals from surrounding atmosphere.

Authors' contributions

All of works related to this manuscript were been done by the author, Mohammad Sanad Abu-Darwish . These including collection of raw materials of studied medicinal plants , Analyzing of heavy metals content , evaluation of obtained results by statistical analysis system , collection of literature data and reviewing of previously obtained results by other authors , and writing the manuscript .

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