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Metal Concentration in Commonly Used Medicinal Herbs and Infusion by Lebanese Population: Health Impact

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Abstract

The levels of K, Ca, Fe, Zn, Cu, Mn, Pb, As, Cd, Cr, and Pb in commonly used medicinal herbs and their infusions by Lebanese were analyzed by EDXRF and ASS techniques, respectively. The order of metal contents in the herbs was found to be: K (6990-19850 µg/g) > Ca(1630-14450 µg/g) > Fe(80-3650 µg/g) > Mn (28-458 µg/g) > Zn (23-108 µg/g) > Cu (5-71 µg/g) > Cr (3.1-55 µg/g) Pb> (1.1-10.3 µg/g) > As (nd-10.8 µg/g) > Cd (nd-1.7 µg/g). The mean levels of toxic metals in herbs Pb, As and Cd were below WHO permissible levels. Cluster analysis indicated metals are most probably in plants due to wastes disposal and irrigation with contaminated wastes and/or from atmospheric waste particulates. The levels of Mn, Cr, Pb and As in herbal infusions were found to be higher in soaked than boiled preparations and correlated with Fe, while Zn and Cu levels were higher in boiled infusions. The highest weekly intake from herbal infusions of toxic metals Cr (492.8 µg), As (77.0 µg), Pb (291.2 µg) and Cd (19.0 µg) were below the recommended permissible tolerable weekly intake respectively 1260 µg, 900 µg, 1500 µg, and 150 µg. Therefore, the consumption of these traditional medicinal herbs does not pose any health risk provided full compliance with recommended daily doses.

Keywords: medicinal herbs, infusion; metals, weekly intake, Lebanon

1. Introducation

The use of plants as medicine predates recorded history despite medical and pharmaceutical advancements. In fact 70-80% of the world's population still primarily relies on non-conventional medications, mostly derived from plant substances (Li, Fang, Ning, & Wu, 2012; Nwoko & Mgbeahuruike, 2011). The use of medicinal plants in primary healthcare intervention is more common in developing countries, where commercial drugs are mostly unaffordable or unavailable; furthermore, traditional medications are readily available and more culturally acceptable (Okatch, Ngwenyas, Raletamo, & Andrae-Marobela, 2012).

The effectiveness of medicinal plants for therapeutic purposes is often accounted for by their chemical constituents like flavonoids, alkaoids, terpenes, glycosides, essential oil, vitamins, etc. (Ashraf, Hayat, & Mumtaz, 2010; Chuparina & Aisueva, 2011). Additionally, minerals and trace metals are partially responsible for their medicinal and nutritional properties, as well as their toxic ones. These elements play an important role in plant metabolism and biosynthesis and as cofactors for enzymes (Maiga, Diallo, Bye, & Paulsen, 2005; Tokalioğlu, 2012). The elemental composition of herbs is a reflection of the environment they grow in. The levels of essential elements in plants vary according to the geographical region, geochemical soil characteristics, and the ability of plants to selectively accumulate some of these elements. Generally, these elements are absorbed through the root systems and dispersed throughout the plant body (Hondrogiannis et al., 2012). Some metals are essential nutrients (zinc, iron, copper, and chromium), yet become toxic at high concentrations, while others (lead, mercury, arsenic and cadmium) have no known beneficial properties and are toxic (Maiga et al., 2005; Gjorgieva, Kadifkova-Panovska, Bačeva, & Stafilov, 2010).

Accumulation of heavy metals in plants is one of the most serious environmental concerns, not because of the phytoxicicity of many of the metals, but also because they transfer heavy metal pollutants from soil into the food chain, and cause adverse health effects in humans (Rao & Galib, 2011). There are a number of factors which contribute to heavy metal contamination in agricultural soils including waste and industrial water discharge, irrigation with contaminated water, fertilizers and pesticides (Nwoko & Mgbeahuruike, 2011). Additional sources of elements in plants are rainfall, atmospheric dust that can be absorbed through leaves (Rao & Galib, 2011; Pytlakowska, Kita, Janoska, & Kozik, 2012). Asian traditional medicines have been reported to contain high levels of heavy metals like arsenic, lead and mercury (Okatch et al., 2012). Heavy metals have also been detected in herbal remedies in South America (Gomez, Cerutti, Sombra, Silva, & Martınez, 2007) and Africa (Obi, Akunuilli, Ekpo, & Orisakwe, 2006). Thus, assessing metals in medicinal plants is important in relation to their safety and in initiating awareness among consumers who often assume that all natural products are safe.

The elemental concentration in medicinal herbs have been measured by various spectroscopic techniques, neutron activation-INAA, X-ray fluorescence (Yuan, Chapman, & Wu, 2010), and energy dispersive X-ray fluorescence-EDXRF (Al-Omari, 2011). Better detection limits and more elements would be obtained using X-ray tubes as excitation sources instead of radionuclide sources. With modern software's, it is possible to produce clean spectra with net peak intensities, and precise corrections for inter-element matrix effects. As a consequence, detection limits less than 1 µg/g were achieved for many elements by EDXRF (Al-Omari, 2011).

Despite recommendations to developing countries to investigate plants used in their traditional medicine as potential sources of new drugs (Samuelson, 1987) , such studies remain meager in many of these countries (Saad, Azaizeh, Abu-Hijleh, & Said, 2006; Saganuwan, 2010; Mati & De Boer, 2011). In Lebanon, research on medicinal plants has mainly focused on the identification and characterization of their essential oils and pharmacologically important secondary metabolites, and studies that compiled inventories of plants used in traditional medicine (El-Beyrouthy, Arnold, Annick, & Frederic, 2008). However, no studies on metal levels in commonly used medicinal herbs in Lebanon have been conducted yet. Therefore, this study will be the first of its kind to produce a baseline data of major and toxic metal levels in sixteen commonly used medicinal herbs, local and imported, and their infusions by the Lebanese population. In this study we used the common practice of herb infusions, and were prepared by either boiling the herb with boiling water or soaking the herb in boiled water to simulate its traditional usage.

2. Materials and Methods

2.1 Samples

Sixteen medicinal herbs or blends were purchased from Dabbous Herb Store, one of the oldest and major herbalists in Beirut, Lebanon. This herbalist is the main one in Lebanon as a whole, with many branches all over Lebanon. Most Lebanese citizens purchase their herbs from this shop. Hence, these sold herbs can be extrapolated to represent the entire country. Upon the purchase, the herbalist on duty was asked about the name of the purchased plants, their country of origin and their medicinal uses. The herbs were identified at least to the genus level using Bailey (1949), and Mouterde (1966). Information pertaining to the plants is presented in Table 1.

2.2 Herb Samples Preparation and Metal Analysis

The herbs were washed with distilled water and air-dried at room temperature. The dried samples were grinded to fine homogeneous powder and stored in plastic bags for metal analysis.

The concentrations of metals (Ca, K, Fe, Mn, Zn, Cu, Co, Ni, Cr, Pb, Cd, As) in the dried herbs was determined using energy dispersive X-ray fluorescence (EDXRF) (Niton XL3 GOLLD hand held, Thermo Fisher Scientific) with up to 50kV x-ray tube source and optimized silicon drift detector (SDD). The efficiency and accuracy of this analytical method was checked by using the certified reference material Tomato Leaves (NIST 1573a) on 4 replicates. The recovery percentages ranged between 83 % and 104 % (Ca: 102%, K: 99 %, Fe: 101%, Mn: 98 %, Zn: 101%; Cu: 94%; Ni: 90%, Cr 89%; Cd: 88%).

2.3 Herbal Infusion Preparation and Metal Analysis

Two types of herbal infusions were prepared based on the common methods used by Lebanese (boiling the herbs with water or by soaking the herbs in hot water). Boiled herbal infusions were prepared by boiling two replicates (four grams) of each dried plant for five minutes in 250 ml of distilled water in a beaker covered with a watch glass. The 4 grams of herbs were used based on citizen customary herbal quantity adopted for their herbal infusion beverage preparation upon the recommendation of the herbalist. Each obtained solution was then filtered using Buchner funnel with Fritted Discs. The solutions obtained from the two replicates were then transferred to a 500 mL calibrated plastic bottle and diluted to mark with distilled water. One drop of concentrated nitric acid was then added to the bottle, which was stored at 4°C for later analysis of metals. A similar extraction and preservation procedure was used for the soaked herbal infusion, except that distilled water was first boiled, then dried plants were soaked in the boiled water for 15 minutes.

The concentration of metals in infusion (boiled and soaked) were determined by using Atomic Absorption Spectrophotometer (AAS)-Graphite furnace except for iron, which was determined by Fe Flame AAS ("Shimadzu" AA-6300) and background correction deuterium lamp. Working standard solutions were prepared by dilution of stock solutions (1 mg metal/ml in 2% HNO_3) with MilliQ water. Standard addition calibration method was used to reduce the effect of the matrix in the infusion.

2.4 Software Used in Analysis

Statistical analyses were performed using Microsoft Excel (Microsoft, 2007), PASW Statistics 18 (SPSS Inc., 2009) and SigmaStat (version 3).

Table 1. Characteristics of used medicinal plants

Code	Local Name	Origin	Scientific Name (Family)	Part Used	Therapeutic Indication
H1	Zhoorat	Lebanon	A mixture of *Alcea setosa* and other *Alcea* spp. (Malvaceae); *Matricaria* sp. and *Anthemis* sp. (Asteraceae); *Rosa damascena* (Rosaceae)	Flowers and leaves	Cold
H2	Zoofa	Lebanon	*Micromeria juliana* (Lamiaceae)	Stems, leaves and flowers	Cough
H3	Ward	Lebanon	*Rosa damascena* (Rosaceae)	Floral buds	Anxiety
H4	Dhanab Al Khayl	Syria	*Equisetum* spp. (Equisetaceae)	Vegetative stems	Osteoporosis
H5	Karkade	Egypt	*Hibiscus* sp. (Malvaceae)	Flowers	Hypertension Hyperlipidemia
H6	Shaqaik Al No'man	Syria	*Anemone coronaria* (Ramuculaceae)	Flowers	Whooping cough
H7	Lsan Al Asfoor	Lebanon	*Ficus religiosa* (Moraceae)	Leaves	Cough
H8	Malleeseh	Lebanon	*Melissa sp.* (Lamiaceae)	Leaves	Anxiety and depression
H9	Yansoon	Italy	*Pimpinella anisum* (Apiaceae)	Seeds	Colic, Insomnia
H10	Qas'een	Lebanon	*Salvia fruticosa* (Lamiaceae)	Leaves	Colic
H11	Zahrat Al Banafsaj	Lebanon	*Viola odorata* (Violaceae; Possibly mixed with *Cercis siliquastrum* (Fabaceae)	Flowers	Atherosclerosis
H12	Kammoon	India	*Cuminum cyminum* (Apiaceae)	Seeds	Colic
H13	Ikleel Al Jabal	Lebanon	*Rosmarinus officinalis* (Lamiaceae)	Stems and Leaves	Hyperlipidemia
H14	Zayzafoon	Lebanon	*Tilia* sp. (Malvaceae)	Leaves	Anxiety and depression
H15	Zahrat al Masy	Lebanon	*Sena* sp. (Fabaceae)	Leaves	Edema
H16	Babounij	Syria	*Matricaria* spp. (Asteraceae); *Anthemis* spp. (Asteraceae)	Flowers	Anxiety Insomnia

3. Results and Discussion

3.1 Metals in Herbs

3.1.1 Concentration of Metals in Herbs

Table 2 presents the mean concentration and SD of each element of all investigated herbs analyzed by EDXRF technique. All samples were analyzed three times.

The highest element concentration in most of the studied plants was potassium, followed by calcium. The mean concentration of K of the different herbs was 12015 μg/g (≈12 mg/g). The highest K level was in sample H16-Babounij (Chamomile-Asteraceae) with a level of 18852 μg/g (≈20 mg/g), and lowest in H13- Ikleel Al Jabal (rosemary-Lamiaceae) with a level of 6985 μg/g (≈7 mg/g). Whereas, the mean concentration of Ca in herbs was 7766 μg/g (7.7 mg/g), and highest in H10-Qas'een (Lamiaceae) with a level of 14448 μg/g (14.4 mg/g) and lowest in H16-Babounij (Asteraceae) with a level 3855 μg/g (≈4 mg/g). Both K and Ca are present in plants in large amounts; they are nutrient elements (Gjorgieva, Kadifkova-Panovska, Baeva, & Stafilov, 2011).The highest K levels for H1-Zhoorat and H16- Babounij (Table 2) of plant family Asteraceae coincide with those found by Gjorgieva et al. (2011), in chamomile with a value of 28178 μg/g (≈28 mg/g). Also, our K levels were similar to those reported by Kara (2009) for chamomile, with 18399 μg/g (18.4 mg/g).

Table 2. Concentration of metals in plants (μg/g) except as specified by * (mg/g)

Code	Ca*	K*	Fe*	Mn	Zn	Cu	Cr	Pb	Cd	As
H1	8.20±0.73	13.11±1.15	0.88±0.20	96 ±10	65±10	43±4	4.6±0.9	3.2±0.2	nd	nd
H2	11.75±1.2	8.67±0.98	0.64±0.16	99 ±19	61±7	29±3	5.0±0.4	2.2±0.1	nd	nd
H3	4.98±0.09	9.89±1.10	1.84±0.52	250±20	55±5	47±3	3.1±0.1	1.1±0.1	nd	6.9±0.10
H4	6.98±0.17	11.06±0.98	0.53±0.11	88 ±9	23±4	20±1	6.4±0.6	4.0±.4	1.4±0.07	nd
H5	5.73±0.09	11.78±0.75	0.08±0.01	334±26	30±5	5±1	4.1±0.2	5.3±0.3	1.0±	nd
H6	1.63±0.03	12.32±1.30	0.85±0.08	59±11	71±10	39±3	8.2±0.3	8.2±0.6	0.9±0.06	nd
H7	2.40±0.08	15.491.45	0.17±0.03	28±3	38±5	20±2	9.0±0.2	4.4±0.1	nd	2.1±0.06
H8	13.30±2.10	12.56±0.85	0.14±0.01	123±20	27±2	30±4	8.3±0.7	3.0±0.2	1.4±0.07	nd
H9	7.92±0.55	19.09±2.76	0.92±0.09	171±22	42±4	37±3	5.9±0.2	3.5±0.2	1.7±0.10	nd
H10	14.45±1.4	7.64±0.75	1.70±0.21	108±14	108±13	59±6	5.0±0.1	10.3±0.7	1.1±0.04	10.8±1.2
H11	6.61±0.15	11.74±0.97	1.11±0.30	83±8	108±10	33±3	9.1±0.3	6.4±0.4	nd	nd
H12	6.82±0.45	16.80±1.88	0.80±0.12	59±6	55±4	28±2	10.3±0.2	2.5±0.1	nd	nd
H13	9.61±0.25	6.99±0.65	2.70±0.65	208±15	51±3	31±2	20.3±7	8.4±0.9	nd	10.1±0.92
H14	8.97±0.66	8.23±0.55	1.52±0.11	159±14	56±7	71±9	8.3±0.2	2.1±0.2	1.2±0.02	4.3±0.06
H15	11.05±0.97	7.04±0.45	3.65±0.71	458±44	61±8	35±3	55.4±14	4.2±0.3	nd	4.4±0.04
H16	3.86±0.15	19.85±1.30	0.42±0.03	71±5	61±10	40±4	9.3±0.3	7.3±0.4	nd	nd

Iron and manganese are considered essential nutrients. The concentrations of iron in studied herbs were high and came second after those of Ca. The mean concentration of Fe was 1118 μg/g. The highest concentration of 3643 μg/g was in H15- Zahrat al Masy (Fabaceae), and the lowest Fe level of 77 μg/g was in H5- Karkade (Malvaceae). Generally, the iron levels in plants are not high compared to soil Fe levels, and plants derive their Fe content in plants from soil. The Fe content in H2-Zoofa, H10-Qas'een, and H13-Ikleel Al Jabal (Table 2) were higher than those reported by Maiga et al. (2005) for similar plant family (Fabaceae), such as in *D. microcarpum* (80 μg/g). Furthermore, Fe content in H12-Kammoon (796 μg/g) was higher than those reported by Hondrogiannis et al. (2012) for Indian cumin (504 μg/g). The high level of Fe in the studied herbs is most probably due to soil particles rich in Fe adhering to the herb. It is recommended to wash the plants with a detergent or acid before the analysis of Fe levels (Campbell & Plank, 1997). However, in our case, the herbs are used by only washing in water (if applicable), since the traditional medical use is through infusion.

The mean concentration of Mn was 149 μg/g with the highest concentration in H15- Zahrat al Masy (Fabaceae), with 458 μg/g. The high Mn could be be most probably due to association of Fe to Mn and adherence of soil particles rich in Fe. Also high Mn concentration (334 μg/g) was found in H5- Karkade (Malvaceae). Our reported levels in H12- Kammoon (59 μg/g) was nearly equal to that in Indian cumin (40 μg/g) reported by Hondrogiannis et al. (2012). Furthermore, a statistical significant correlation ($r = 0.63$, $p < 0.01$) occurred between Fe and Mn in the investigated medicinal herbs. This correlation is most probably due to Fe and Mn's geochemical association in soils. Both Fe and Mn are considered as reducible geochemical fraction in soil (Korfali & Davies, 2004; Korfali & Jurdi, 2010).

Zinc and copper are essential micronutrients; they are essential trace metals for plant growth and development (Hussain, Khattak, Ali Khan, Rehman, & Ullah Khan, 2011). Phytoxicity of copper can occur if its concentration in plants is higher than 20-100 μg/g DW (dry weight). All Cu levels of samples fell within this interval, except one sample was even below (5 μg/g). Zinc is not considered to be highly phytotoxic, but high contents of Zn in plants may cause the loss of leaves' production (Gjorgieva et al., 2011). The mean concentration of Zn in the investigated herbs was 57 μg/g. The highest concentrations of 108 μg/g were found in H10-Qas'een (Lamiaceae), and H11-Zahrat Al Banafsaj (Violaceae), and these herbs are leafy and flowery. The lowest Zn level of 23 μg/g was found in H4- Dhanab Al Khayl (Equisetaceae), vegetative stems. The high level of Zn is most probably related to waste water discharge and irrigation with waste water and/or atmospheric fall out (Nwoko & Mgbeahuruike, 2011; Rao & Galib, 2011) that render leaves and flowers to be high in zinc. Compared to other works, our reported Zn levels were higher than those of Li et al. (2012) of 32.64 μg/g for Radix Salvia Miltitorrhiza root part (Lamiaceae). This discrepancy in Zn levels of herbs belonging to similar plant family may be due to the difference in the part of herbs under investigation where the latter was performed on the roots while ours on the leaves and flower (878 μg/g) that are more exposed to waste water discharge and irrigation with waste water and/or atmospheric fall out. As for copper, the mean concentration was 35.47 μg/g, with the highest concentration in H14-Zayzafoon (Malvaceae) of 71 μg/g, and then in H10-Qas'een (Lamiaceae) of 59 μg/g (both herbs are consumed as leaves). Similar to Zn, the high levels of Cu in these plants are the outcome of nearby waste water discharge or irrigation with waste water, and due to pesticides. In fact Cu is a constituent used in pesticides (Tokalioğlu, 2012). A statistical significant correlation ($r = 0.62$, $p < 0.01$) occurred between the levels of Zn and Cu in the investigated medicinal herbs. This reflects the association of these metals in plants due to the physiology of metal association, and/or similar geochemical association. Both metals have geochemical properties associated with the organic soil chemical fraction (Selinus et al., 2005).

Chromium is an essential micronutrient, but it becomes toxic at high levels, and chronic exposure to Cr may result in liver, kidney and lung damage (Tokalioğlu, 2012). The mean concentration of Cr in investigated herbs was 10.56 μg/g. The highest Cr level of 55 μg/g was in H15-Zahrat al Masy (Fabaceae) and then in H13-Ikleel Al Jabal (Lamiaceae) of 20 μg/g. The leaf is used in both previous mentioned medicinal herbs. The high reported levels of Cr in our H13 and H15 samples is most probably due to the geochemical association of Cr with Fe (Korfali & Jurdi, 2010) , where both H13 and H15 samples had high Fe levels. Consequently, Cr has accumulated in plants by adsorption of Cr to Fe oxides and hydroxides in soil particles. A statistical significant correlation ($r = 0.84$, $p < 0.01$) occurred between Cr and Fe in investigated herbs.

Lead, arsenic and cadmium are of no nutrient value and are toxic trace elements with adverse health effects (Nwoko & Mgbeahuruike, 2011; Tseng, Chong, & Tseng 2003). The maximum permissible levels in raw medicinal herbs for Pb, As, Cd are respectively 10 μg/g, 5 μg/g, and 0.3 μg/g (WHO, 2007).

In this investigation the mean level of Pb was 5.4 μg/g, the highest level of 10 μg/g was in H10-Qas'een (Lamiaceae). All samples had permissible limit of Pb in medicinal herbs, with H10 reaching the borderline. However, H10 may be considered to pose health problem, and consumption of this herb should be cautious. Our data about Pb levels were lower than those of Alomary, El Jamal, Attiyat and Obeidat (2012) in medicinal herbs consumed in Jordan, with reported value for Mairamiyeh (Qas'een) of 15.8 μg/g, Yansoun of 8.0 μg/g, and Karkade of 6.8 μg/g.

The mean level of As was 2.4 μg/g, and 10 of the samples (62.5%) were below detection limit (ND) < 1 μg/g. The highest level of 10.8 μg/g is found in H15-Zahrat al Masy (Fabaceae), in H13-Ikleel Al Jabal (Lamiaceae), with a value of 10.1 μg/g, and in H3-Ward (Rosaceae), with a value of 6.9 μg/g. These levels are higher than WHO limit of As (5 μg/g) and these herbs should be consumed cautiously.Those samples exhibited also high levels of Fe. High levels of As in food and herbal medicine can be due to its abundance in nature (WHO, 2007). Statistical significant correlation existed ($r = 0.61$, $p < 0.05$) between As and Fe. Similar to the occurrence of Cr in plants, and due to Fe geochemical association properties, the association of Fe with As from soil adhesion to plant occurred. However, the occurrence of As is not only due to natural causes, but might also have been increased through the use of pesticides, where samples with high As are either leaves or flowers.

The mean level of Cd was 0.55 μg/g and higher by a factor of 1.8 more than WHO limit (0.3 μg/g). However, 9 of the samples (56%) were below detectable levels (ND). The highest Cd level of 1.7 μg/g was in H9- Yansoon (Apiaceae) and higher than WHO limit by a factor of 5.6, H8 -Malleeseh (Lamiaceae) andH4- Dhanab Al Khayl (Equisetaceae), in both Cd levels were 1.4 μg/g and higher than WHO limit by a factor of 4.6. Therefore these herbs should be continuously monitored as they pose a very high health risk factor. But, the Cd levels in our samples were lower than those of Subramanian, Gayathri, Rathnavel and Rj (2012) in Indian herbs, with a range between 0.72 and 2.52 μg/g. Cadmium did not show any correlation with any of the studied element. Thus its occurrence is mainly due to contamination factors, such as irrigation with polluted water, wastewater discharge, and/or fertilizers. Nevertheless, we cannot assume that the herbs with high levels of Cd represent health hazards, since these herbs are used as infusion, and Cd in liquids depends on the physiology of the plant and the efficiency of extracting Cd, as well as its adsorption by the body.

3.1.2 Cluster Analysis

Cluster analysis involves trying to determine relationships between objects (samples) without using prior information about these relationships. The raw data for cluster analysis consists of a number of objects and related measurements. Objects will be grouped in clusters in terms of their nearness or similarity. The cluster analysis was applied using the SPSS package. The measurement is based on the square Euclidean distance; the Wards method was used as clustering method.

From the Dendrogram (Figure 1) we could conclude the following: More homogeneity of clusters being combined is reflected in the shortest rescaled distance of Dhanab Al Khayl (H4-vegatation), Zahrat Al Banafsaj (H11-Flowers), and Karkade (H5-flowers). These associations and clustering indicate that metals are most probably in plants due to wastes disposal and irrigation with contaminated wastes and/or from atmospheric waste particulates. Thus an intervention plan should address the control of disposal and irrigation with contaminated wastewater to reduce the absorption of contaminants by plants and its respective health hazards. Next in clustering are Yansoon (H9-seed), Kammoon (H12-seed), Ikleel Al Jabal (H-13-leaves) and Zayzafoon (H14-leaves) which reflects the similarity in the physiology of these plants and their metal absorption abilities. On the other hand, the largest rescaled distance reveals dissimilarity in Shaqaik Al No'man (H6-flowers) and Zahrat al Masy (H15-leaves). This suggests that the similarity in metal content is related to the physiology of the plant mainly affected by waste discharges and atmospheric deposition on plants.

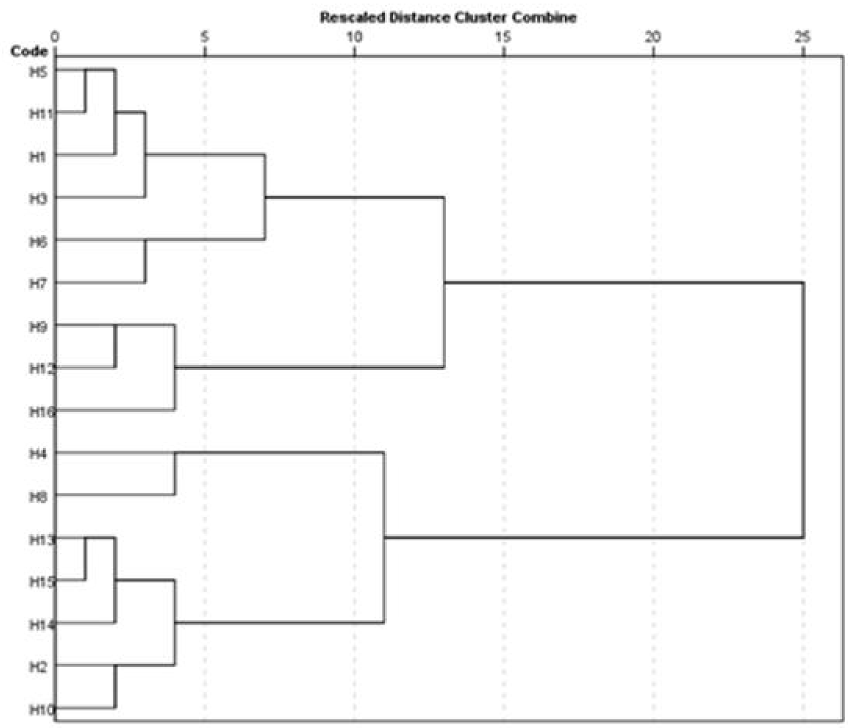


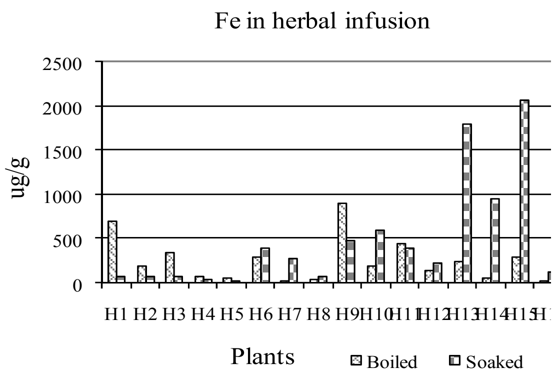
Figure 1. Dendrogram of cluster analysis

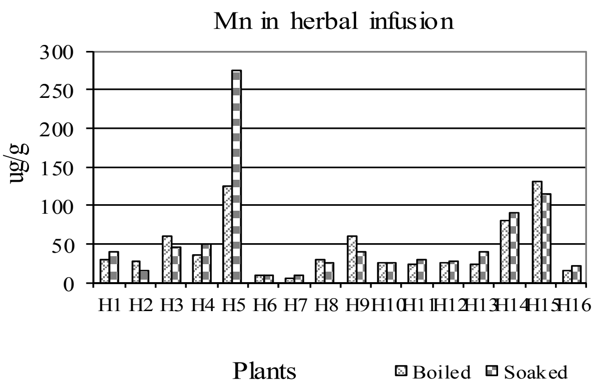
3.2 Concentration of Metals in Herbal Infusion

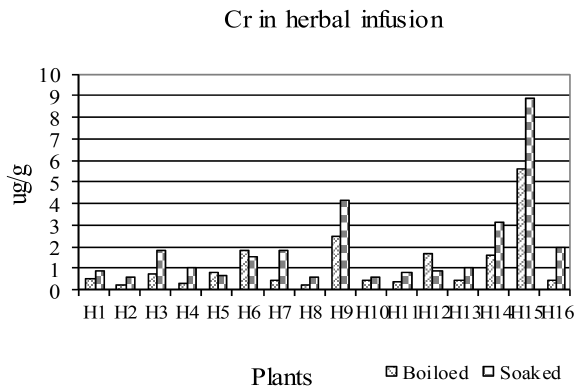
Table 3 presents the mean and range of metal concentration in herbal infusion prepared by two methods and coded as boiled or soaked. The concentration of these metals (Fe, Mn, Zn, Cu, Cr, Pb, Cd, As) were determined to assess the actual metal quantity the citizen is exposed to through the consumption of these herbal infusions as beverages and/or medications.

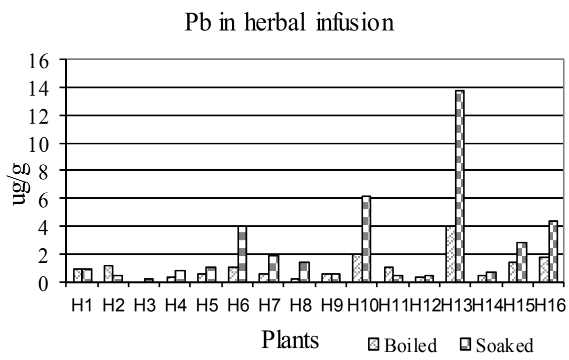
Table 3. Mean and range concentrations of metals in herbal infusion

Metal	Boiled			Soaked		
	Mean	Range	% Range extracted	Mean	Range	% Range extracted
Fe (mg/L)	3.67	0.32-14.19	3-48	7.44	0.12- 32.69	3-60
Mn (mg/L)	0.67	0.03-2.11	5-45	0.84	0.09-4.49	12-70
Zn(μg/L)	235	7-642	1-50	81	7-494	1-30
Cu(μg/L)	129	9-263	1-50	52	7-191	1-18
Cr(μg/L)	17.4	2.4-88	2-40	29.8	8-140.8	5-70
Pb(μg/L)	16.5	5.6-63.8	8-60	39.8	8.0-218.4	8-70
Cd(μg/L)	2.96	0.10-8.40	8-25	3.69	0.11-6.34	10-35
As(μg/L)	4.29	0.15-16.50	1-70	5.74	0.10-21.99	5-50









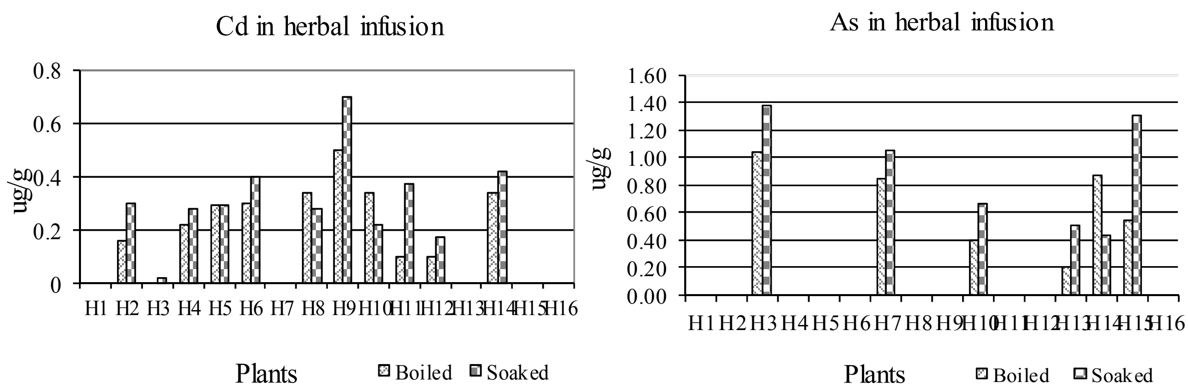


Figure 2. Metals of higher concentrations in soaked than boiled herbal infusion

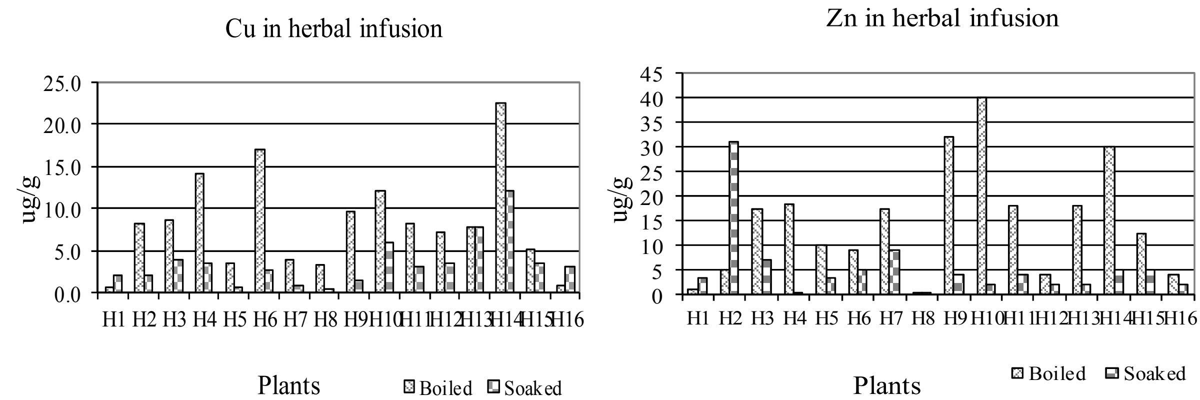


Figure 3. Metals of higher concentrations in boiled than soaked herbal infusion

It is evident from Table 3 and Figures 2 and 3 that for most metals, except Zn and Cu, the leaching of metals from herbs into the solution is higher in soaked infusion than boiled. Furthermore, metals (Mn, Cr, Pb, As) of higher concentrations in soaked herbal infusion are the ones correlated in herbs with Fe. Also, the One Way Analysis of Variance (ANOVA) of the different samples revealed similar to Fe, no statistical significant differences between metal transfers from herbs, whether the herbs are soaked or boiled. The significance level for: As and Mn $P = 0.454$, Cr $P= 0.366$, Pb $P = 0.201$, Cd $P = 0.302$. Whereas, statistical significant differences occurred for Zn ($P = 0.021$) and Cu ($P = 0.001$). Thus, these differences in levels of metals whether soaked or boiled may have resulted from the association of these metals in plants (herbs). Most probably Zn and Cu are associated or form complexes with organic constituents in plants, and accordingly organic constituents are more oxidized in the boiled infusion thus releasing Zn and Cu. The association of Zn and Cu to organic constituents is well documented (Madejon, De Mora, Felipe, Burgos, & Cabrera, 2006). Whereas, Fe is most probably present in plants in exchangeable forms that were released gradually into the solution driving out with it metals associated to Fe. While through boiling, iron is released as a precipitate, hence reducing its level in the boiled infusion herbs.

The percentage levels of iron in herbal infusion from total levels in herbs is lowest compared to other metals (Table 3). The percentage extracted in solution ranges between 2.5 and 20% (mean 8.8%) for boiled herbal infusion, but between 3.5 and 38% (mean 16.5%) in soaked herbal infusion. The differences in the amount of Fe extracted into herbs infusion depends principally on whether the compound is strongly bound to the matrix or more soluble in solution employed (Pytlakowska et al. 2012). The highest Fe extracted from boiled infusion (213 µg) was in Zahrat Al Banafsaj (H11), a flower, and in soaked herbal infusion, levels of Fe reached 944 µg/g (35% leached) in Ikleel Al Jabal (H13), partly leafy. Both herbs (flower, leaf) indicate surface Fe leaching, loosely bound and could be due to Fe fine soil particles. Furthermore, our Fe in herbal infusion would be classified as a poorly extractable element (< 20 %) as indicated by Pytlakowska et al. (2012).

The percentages of Mn extracted in herbal infusion were higher than those of Fe, with a range between 5 and 45% for boiled herbal infusion, and a higher percentage in soaked herbal infusion, ranging between 14 and 70%. The highest percentage of Mn extraction in boiled herbal infusion was in Zayzafoon (H14), leafy herbs and a level 71.6 µg/g. In soaked infusion Mn level in Karkade reached 234 µg/g which is twice its level in boiled infusion. Manganese has been classified as moderately extractable (22-50%) by Pytlakowska et al. (2012). Yet, in Karkade the extraction of Mn attained 70%, which in this case is classified as highly extractable. The Mn high extraction efficiency in Karkade might be due to a weak binding ability in herbs and a high soluble chemical forms. Thus, the differences in the extracting efficiency of the different herbs reflect their ionic and covalent characteristics, their chemical and biological behavior in herbal leaves together with their solubility (Pytlakowska et al., 2012). Manganese is an essential element incorporated into a number of metaloenzymes and the recommended daily allowance is between 2 and 5 mg. (Mehra & Baker, 2007).Thus drinking 2 cups of Karkade per day provides the minimal recommended daily allowance of Mn. But, the contribution of Karkade to mineral absorption is ill-defined and further study of this aspect is needed.

The extraction efficiencies of both Zn and Cu were almost the same in herbal infusion. This extraction efficiency is higher in boiled infusion than in soaked. The percentage ranges of extracted Zn and Cu in boiled infusion were between 1 and 50%, with a mean of 17% .While Zn in soaked infusion ranged between 1 and 30% (mean 5.6%) and Cu between 3-18% (mean 6.25 %). Zinc and Cu can be classified as poorly extracted elements in studied herbs since their mean percentage is $<20\%$ (Pytlakowska et al., 2012). These findings suggest that Zn and Cu are strongly bound to organic constituents in herbs and are difficult to extract under normal conditions. The higher percentage extraction of Zn and Cu in boiled herbal infusion is most probably due to a higher oxidation rate of organic herbal content at higher temperatures, which leads to a higher digestion rate. The highest percentage of extracted Zn from herbs by boiling was in Yansoon- H9 (45%). This high percentage of extraction might be due to the larger surface area of Yansoon seeds that induce further extraction. For Cu, the highest percent extracted was in Dhanab Al Khayl-H4 stems (18%). Our Zn and Cu percentages extraction in herbal infusion was lower than those of Shen and Chen (2008) for Zn with a range between 52.5 and 60.7%, and Cu was lower than those of Street, Drábek, and Mládková (2006) with 26%. Therefore, the element concentration in different herbal infusion may be affected by different physiological properties or structures of plants, such as the levels of phytochelating and binding compounds, and most significantly the solubility of these elements in water (Zhu, Wang, Fan, Qiao, & Yao, 2012).

The mean percentage extraction of Cr in herbal infusion was low, in boiled (12%) and in soaked infusions (21%). However, there was a wide percentage extracted range in the different herbal infusions. In soaked herbal infusions the range extended between 5 and 70%, and in boiled infusion between 2 and 40%. The highest concentration of Cr was in H15-Zahrat al Masy (8.8 µg/g) soaked herbal infusion. The Cr levels were correlated to Fe levels. The high percentage of Cr in some herbal infusion is mostly related to plant physiology and/or Cr in plants in exchangeable sites associated with Fe soil particles

Analogous to Cr, both Pb and As the percentage extraction in soaked preparations were higher than those in boiled infusion. The mean Pb extracted in soaked infusion was 37%, and ranged between 15 and 70%, and in boiled infusion, the mean percent Pb was 20 and ranged between 8 and 60%. The highest extracted Pb in boiled infusion (60%) was in Zoofa (H2), and in soaked infusion in Zahrat al Masy (H15) (70% extracted), yet the levels of Pb in the solution was still within the norms (2.8 µg/g) due to the low content of Pb in this herb. Though, Qas'een (H10) had a lower extracted percent (62%), yet the levels of Pb in solution were higher (6.2 µg/g), due to Pb higher level in this herb (10 µg/g). All of these herbs were used as leaves. The high transport of Pb from plant to solution is mainly at the leaf surface and most probably due to atmospheric deposition that rendered Pb to be in exchangeable form in plant. Whereas, the levels of infused As were low in both boiled (nd to 1.00 µg/g) and soaked infusions (nd-1.37 µg/g). The highest As extracted percent was in Lsan Al Asfoor (H7) in both soaked (50%) and boiled (40%) infusions, still the levels of this metal in plants were low (2.94 µg/g). However, herbs with the highest As levels ($\cong$ 10.5 µg/g) Zahrat al Masy (H15) and Ikleel Al Jabal (H13) had low infusion As levels because of the low percent transfer from plants of 2 and 5% respectively.

Finally, the mean of extracted Cd levels in herbal infusion (boiled and soaked) were low and similar ($\cong$ 7%), and comparable in range (nd-25%). The highest extractable Cd was in Yansoon (H5) which are fine seeds with a large surface area and in this seed Cd is most probably bound to a surface exchangeable site sustaining high transfer capacities. Our extracted percentage of Cd in Karkade (15%) was lower than that of Arpadjan,Çelik, aşkesen, and Güçer (2008), with a level of 74%. A plausible explanation for this difference could be due to the quality of herbs sold mixed with other herbs hence not totally representing the herb under investigation.

3.3 Weekly Intake of Toxic Metals

Most of traditional herbal medications are prepared from dried herbal material, using the dried herbs or ground powder for water infusions. Based on this commonly used treatment regime, the possible maximum weakly intake of Cr, Pb, As, Cd were calculated assuming 2 cups of daily consumption of an infusion, which is the custom followed for all the studied herbs, and prepared from 4 g dried plant in a volume of 250 mL of water (1 cup), as indicated in the section of Material and Methods. Generally, the consumption of two cups is the practice adopted by the citizen, though it could reach by some to 4 cups of herbal infusing in a day. Then, the weekly intake is determined from reported results of each metal intake in a 2 cups (500 mL) multiplied by 7 (days). These values were compared with maximum provisional tolerable weekly intake (PTWI) values (Table 4) recommended by health international organizations and considering 60 kg body weight.

Table 4. Weekly intakes of toxic metals compared to permitted intake

Plant	As			Cd			Pb			Cr		
	Weakly Intake (μg)		PTWI[a,b] (μg)	Weakly Intake(μg)		PTWI[a,c] (μg)	Weakly Intake (μg)		PTWI[a,d] (μg)	Weakly Intake (μg)		PTWI[a,e] (μg)
	B[f]	S[g]		B[f]	S[g]		B[f]	S[g]		B[f]	S[g]	
Zhoorat	-	-		-	-		25.2	25.2	1500	11.2	4.5	1260
Zoofa	-	-		-	-		33.6	12.3	1500	4.2	14	1260
Ward	28.8	38.5	900	-	-		2.8	5.04	1500	16.8	46.2	1260
Dhanab Al Khayl	-	-		3.1	3.9	150	9.0	22.4	1500	8.4	26.9	1260
Karkade	-	-		4.2	4.2	150	16.8	29.4	1500	22.4	16.8	1260
Shaqaik Al No’man	-	-		4.5	5.5	150	29.1	112	1500	51.5	42.6	1260
Lsan Al Asfoor	23.5	29.3	900	-	-		15.7	53.8	1500	24.2	50.4	1260
Malleeseh	-	-		4.7	3.9	150	7.6	37.8	1500	6.7	15.7	1260
Yansoon	-	-		7.1	9.5	150	14.7	14.7	1500	67.2	117.6	1260
Qas’een	11.1	18.4	900	7.7	5.5	150	56.2	173.6	1500	12.6	15.4	1260
Zahrat Al Banafsaj	-	-		-	-		30.2	13.4	1500	10.1	22.7	1260
Kammoon	-	-		-	-		9.8	14	1500	42	25.2	1260
Ikleel Al Jabal	5.7	14.2	900	-	-		42.6	145.6	1500	11.2	28	1260
Zayzafoon	24.2	12.1	900	5.0	6.0	150	11.8	19.6	1500	44.8	89.6	1260
Zahrat al Masy	15.2	36.4	900	-	-		38.1	78.4	1500	154	246.4	1260
Babounij	-	-		-	-		51.0	121.5	1500	12.6	55.44	1260

[a] Provisional tolerable weekly intake ;

[b]Calculated from 15 μg/kg bw (NSF 2003) for 60 kg body weight;

[c]Calculated from 2.5 μg/kg bw (EFSA 2009)for 60 kg body weight;

[d]Calculated from 25 μg/kg bw (JECFA 2000)for 60 kg body weight;

[e]Calculated from daily intake of 180 μg for 60 kg body weight (NSF, 2003) multiplied by 7;

[f] Boiled herbal infusion;

[g] Soaked herbal infusion.

The highest weekly intake was for: As 77 µg in Ward (H3), Cr 492.8 µg in Zahrat al Masy (H15), Pb 347.2 µg in Qas'een (H10), Cd 19.0 µg in Yansoon (H9). The provisional tolerable weekly intake (PTWI) is as follows: As is 900 µg, and amount of intake from Ward (H3) is 8.5% its PTWI; Pb is 1500 µg, and amount of Pb intake from Qas'een (H10) is 23% of its PTWI; Cr is 1126 µg, and the amount intake from Zahrat al Masy (H15) is 38% of its PTWI; Cd is 150 µg, and the amount intake from Yansoon (H9) is 12.6% its PTWI. Therefore, the consumption of commonly sold traditional medicinal herbs in Lebanon does not present any health hazard or poses any health risk factor. Yet the citizen should be aware and advised by the herbalist not to consume these herbs in excess, since citizens assume that these herbs are safe and can be consumed in any amount. Still, from this study draw the attention of the government to a continuous monitoring of the quality of herbs sold in the Lebanese market and set Rules and Guidelines governing the manufacturing or importing herbal products.

4. Conclusion

(1) The high concentration of Fe in herbs were most probably due to adhesion of rich soil particles on herbs

(2) Though, the mean levels in herbs of toxic metals Pb 5.4 µg/g, As 2.4 µg/g, and Cd 0.55 µg/g were below WHO permissible levels. Yet, one sample was higher than WHO Pb limit, 3 samples were 2 times higher than WHO As limit, and 7 samples were far above WHO limit for Cd.

(3) Cluster analysis indicated metals are most probably in plants due to wastes disposal and irrigation with contaminated wastes and/or from atmospheric waste particulates.

(4) The infusion levels of Mn, Cr, Pb, As were higher in soaked than boiled infusion and were correlated with Fe that regulated their association in herbs

(5) Zn and Cu levels were higher in boiled infusion and most likely were regulated by organics

(6) The highest weekly intake of toxic metals from herbal infusion calculated from most used traditional method was for Cr 492.8 µg, As 77 µg, Pb 347.2 µg, Cd 19.0 µg, and were below recommended permissible tolerable weekly intake.

(7) The consumption of sold traditional medicinal herb infusion in Lebanon does not appear to pose a health risk factor by metals. However, we may not assume these herbal infusions do not pose health hazard that might result from fungicides, pesticides, fertilizers, and organics in dumped wastes and irrigation by contaminated wastewater. Further assessment of these should be conducted to fully assess the quality herbs as infusion consumption.

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