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Trace Metal Determination in Herbal Plants by Acid Digestion From Jeddah Market in Saudi Arabia

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Abstract

The world is facing a serious issue with plants contaminated by trace metals. Therefore, a consideration is required due to its danger that impacts both humans and animals. Herbs are extensively used worldwide for their seasoning and therapeutic properties. This study aimed to estimate the level of trace metals (Fe, Zn, Pb, Cr, Cu, Ni) in selected customary herbs consumed in Saudi Arabia. The 5 samples of herbs were purchased from a local market in Jeddah City (Mint (*Mentha)*, Basil (*Ocimum)*, Arugula (*Eruca sativa*), Coriander (*Coriandrum sativum*) and Parsley (*Petroselinum crispum*)). Acid digestion was applied to the plant leaves and trace metals concentrations were determined using Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES). Metals were observed to be available in varied concentrations in the herb plant samples. The highest metal values, especially in Arugula (ES) 218.3 ±1.9 mg/kg and 24.4 ±0.09 mg/kg for Zn and Ni respectively, Cr was under detection limit, Coriander (CS) 148.5 ±1.8 mg/kg and 17.3 ±0.07 mg/kg for Fe and Pb respectively, Mint (ME) 28.6 ±0.26 mg/kg for Cu, while Basil (OC) was recorded below the (WHO) permissible limits 18.9 ± 0.06 mg/kg and 1.1 ± 0.003 mg/kg for Zn and Cr respectively, besides all metals were higher than the (WHO) allowed limit in Parsley (PC). The study found that most of the examined herbs contained hazardous levels of trace metals that exceeded the World Health Organization (WHO) permissible limits.

Keywords: herbal plants, trace metals, acid digestion, determination, ICP-OES

1. Introduction

Heavy metals found in trace amounts in plants, which has a biological effect can be toxic to plants and human being through the food chain. In this report the term “trace metals” will be for these possible phytotoxic elements (Rascio and Navari-Izzo, 2011). The most significant sources of trace metals in the nature are the anthropogenic activities, for example mining, steel, iron or chemical industry, smelting method, traffic, farming as well as domestic activities (Suciu *et al.*, 2008). Plants have ability to absorb and accumulate xenobiotic can be helpful markers of environmental contamination (Farago, 1994; Pandolfini *et al*., 1997).

There are 14 mineral elements are required for the plants in their full physiological activity. Depending on the element, the range of concentration is from 10,000 mg/kg ≥ 0.001 mg/kg (Wheal *et al*., 2011). Every nutrient element is essential within an ideal range. If this range exceeded the possible results may vary from changes in many physiological processes occurring at the cellular/ molecular level such as disabling enzymes, blocking functional groups of metabolic important molecules, substituting essential elements or disrupting the integrity of the membrane (Rascio and Navari-Izzo, 2011). Herbal plants represent an important class of various traditional medicine systems and, in recent years, they are increasingly used in the primary health care intervention in both developed and developing countries. Herbal medicines are extensively used for the treatment of numerous illnesses. They are frequently containing highly effective pharmacological components, including minerals and trace metals (Fabricant and Farnsworth, 2001).

In parallel with the increasing attention of the therapeutic benefits of herbal plants, there is a high concern about the safety and poisonous of natural herbs and formulation given out in the market. There is a widespread misconception that natural herbs and plants are inherently safe. Nevertheless, there has been a large volume of reports on incidences of toxicity and adverse effects linked to the use of herbal plants and their formulations in different parts of the world (AlBraik et al., 2008). The toxicity of herbal plants may relate to contaminants in soil and water such as pesticides, microbes, and chemical toxins also transportation and storage can affect the quality of herbs due to contaminant air (Ernst, 2002; Saad *et al*., 2006). The toxicity of trace metals on human health and the environment has attracted considerable attention in recent years. Plants are the main link in the transferring the trace metals from the contaminated soil to humans through the food chain. Trace metals have low excretion rates through the kidney which could result in damaging effects on humans even at very low concentrations. The essential nutrient metals are Zn, Co, Fe, and Cr, they are important for the physiological and biological functions of the human body. However, an increase in their intake above certain permissible limits can become toxic (Korfali *et al*., 2013). In general, several health problems were linked to excessive uptake of dietary trace metals including a decrease in immunological defenses, cardiac dysfunction, fetal malformation, impaired psychosocial and neurological behavior, gastrointestinal cancer, and many others (Singh *et al.*, 2011; Mahan *et al.,* 2017). Various examination have demonstratd the trace metal contamination of herbel, vegetabls plants and canned fruits collected from market or farms in Saudia Arabia and different countries (Al-Hammad and Abd El-Salam 2016; Maghrabi, 2014; Ali, and Al-Qahtani, 2012; Kananke *et al*., 2014; Begumi *et al*., 2017; Dghaim *et al*., 2015; Massadeh & Al-Massaedh, 2018).

Hence, many techniques were applied for elemental analysis. The most widely recognized strategies utilized nowadays for determining the trace elements in ecological samples include very sensitive spectroscopic methods, for example, inductively coupled plasma-optical emission and mass spectrometry (ICP–OES) and (ICP–MS) in addition to atomic absorption spectroscopy (FAAS, ETAAS) (Wheal *et al*.; 2011). The utilized sample digestion methods are performed by fusion or wet procedure of heating acid mixtures. Various types of heating systems can be utilized, for example, hot plate, sand bath, aluminum blocks and digestion pressure bomb (Altundag and Tuzen, 2011). There is little information available on the safety of traditional herbs and their production seller in the Saudi Arabia market. This study aims to determine the level of trace metals in some commonly consumed herbs; they were purchased from local market in Jeddah City to assess their relative safety and potential health risk based on the World Health Organization standard limits (WHO, 2011; Onder *et al.*, 2007).

2. Material and Methods

2.1 Sample Collection and Preparation

Six samples of each of the different five herbal Plants, which are Mint (*Mentha)*, Basil (*Ocimum)*, Arugula (*Eruca sativa*), Coriander (*Coriandrum sativum*) and Parsley (*Petroselinum crispum*) were collected in December 2016 from local market in Jeddah City, Saudi Arabia (Table 1). First, the plant samples were washed with tap water for removing all dust and impurities until it became cleaned as the last wash was by distilled water. The second step was drying samples at room temperature for few days. Finally, the dried plants were grinding using a grinder and stored in plastic bags.

Table 1. Local and botanical name of the herbs and their code

Local name	Botanical name	Code	Parts analyzed
Mint	*Mentha*	*ME*	Leaves
Basil	*Ocimum*	*OC*	Leaves
Arugula	*Eruca sativa*	*ES*	Leaves
Coriander	*Coriandrum sativum*	*CS*	Leaves
Parsley	*Petroselinum crispum*	*PC*	Leaves

2.2 Acid Digestion Procedures

0.5 g of each plant sample was digested in 50 ml beaker and then 8 ml of concentrated Nitric acid HNO_3 (PanReac 65%) was added, covered with a watch glass and left overnight at room temperature in a fume cupboard. 2 ml of H_2O_2 (Sigma-Alorich 30%) was added in the second day and heated on a hot plate (90 ^oC-100) . When the mixture was nearly dry, 5 ml of distilled water was added and covered to continue the heating process until a light yellowish color fume is produced. 5 ml of deionized water was added and left to cool. The samples were filtered with a hardness filter paper No.2 Whatman and placed in a 50 ml volumetric flask filled up to the mark with 0.1M Nitric acid and kept for analysis (Maghrabi, 2014).

2.3 Sample Analysis

Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) - Perkin Elmer model 7000 DV) was used for analysis of trace metals concentration such as Fe, Cu, Zn, Pb, Ni and Cr in digested plant samples. The parameters of ICP-OES were wet plasma aerosol type of axial view, nebulizer start-up instant condition, flow rate (Ar) 15 L/ min, auxiliary flow (Ar) 0.2 L/min, nebulizer flow (Ar) 0.8 L/ min, sample uptake rate 1.5 mL/ min, and sample flush time 5 Sec. The wavelengths used for observed elements were 206.2 nm for Zn, 259.94nm of Fe, 324.75nm of Cu, 217nm Pb, 231.6 nm Ni, and 283.56nm of Cr. The trace metals concentration was the mean of triplicate readings ±SD of the dry sample. The instrument is daily calibrated using the Merck standard solution to build up trust in the precision, reproducibility as data validation. The following formula was used for calculation.

$$\textbf{Metals}(\textbf{mg/kg}) = \frac{[\boldsymbol{Conc. of\ metals}(\boldsymbol{mg/kg}) \times \boldsymbol{Volume\ of\ sample}\ (\boldsymbol{L})}{[\boldsymbol{Sample\ weight}\ (\boldsymbol{kg})]} \qquad (\mathbf{1})$$

The contamination factor (CF) was used in the literature for assessment the pollution (Hakanson, 1980; Wang *et al*., 2015; Nouri, and Haddi, 2016)). The calculation of CF was from the ratio of the concentration in herbal plant (C_{sample}) divided to the background ($C_{background}$) obtained based on the permissible limit of the metal in the plant by World Health Organization Recommended (WHO) (WHO, 2011). The classification of CF values stated by Hakanson (1980) is: CF<1 entitles low pollution factor, 1≤ CF<3 moderate pollution factor, 3≤ CF>6 is considered a pollution factor, CF ≥ 6 is an abundant pollution factor.

$$\mathrm{CF} = \frac{C_{\mathrm{sample}}}{C_{\mathrm{background}}} \qquad (2)$$

2.4 Statistical Analysis

The mean concentration was subjected to statistical analysis using one-way ANOVA, to test the difference between trace elements in the tested herbal plants, also the Post Hoc tests were applied to measure the significant different between the trace elements for each two elements (significance $P < 0.05$), using the (SPSS 18.0, USA) and Microsoft Excel 2013 software packages.

3. Results and Discussion

The average concentrations of Fe, Cu, Zn, Pb, Ni. and Cr in Parsley (PC), Arugula (ES), Coriander (CS), Mint (ME) and Basil (OC) herbal plants collected from different local market in Jeddah City are shown in (Figure 1) compared with the World Health Organization (WHO) recommended values. The arrangement order of trace metal in the herbal plants was found as in this sequence of Zn > Fe > Ni> Cu > Pb > Cr.

Fe concentration in the herbal plants samples in this study shows a range of values from 44.4±0.15 mg/kg to 148.5±1.8 mg/kg as shown in (Figure 1). Moreover, the Fe amount in almost all plant samples is above the permissible limit prescribed by WHO (20 mg/kg). Fe is a standout element amongst the critical components those are essential to the human body for purpose of oxygen rotation in the blood. Lack of Fe can cause different sorts of illnesses. However, the growth of plants can be affected by high amount of Fe element. The recorded amount of Fe in these herbal plants compared with the amounts of Fe found in Egyptian spices and medicinal plants are ranged between 26.96 mg/kg and 1046.25 mg/kg, while leafy vegetable in Saudi Arabia were found 543.2 µg/g and 399.1µg/g (Abou-Arab and Abou Donia, 2000; Ali and Al-Qahtan, 2012). The clarification of this circumstance is that the uptake of Fe can be aggregated in the leaves, which considered nutrition manufactures within the plants.

The concentration of Zn is 18.9 ±0.08 mg/kg in OC and 218.3±1.9 mg/kg in ES. In the other herbal plants Zn concentration is about of 57±0.06 mg/kg, which is slightly above the permissible limit reported by WHO (50 mg/kg). However, zinc is an essential trace element in human diet; also, Zn is necessary for proper growth, and protein and DNA synthesis (Ogundele et al., 2015). Little information is available on Zn's toxicity; however, high zinc intake beyond permissible limits produces toxic effects on the immune system, blood lipoprotein levels (Ulla et al., 2012)**.** The results agree with the study obtained by Al-Hammad and Abd El-Salam (2016) for Zn level in leafy green (57.50 mg/kg) in Al-Kharj region, Saudi Arabia (Al-Hammad and Abd El-Salam, 2016).

The concentration range of Cr metal in the herbal plants samples is from 4.7±0.08 mg/kg to not-detectable in ES, as shown in (Figure 1) while ME, CS, OC and PC are 2.3±0.002 mg/kg, 2.1 ± 0.003 mg/kg, 1.1±0.003 mg/kg, and 4.7±0.08 mg/kg, respectively. The Cr level is found to be higher than the allowable level which is 1.30 mg/kg according to WHO recommendation. The high Cr concentration in the herbs might be a result of distinctive of different species (Cr^{3+} and Cr^{6+}) which are coming from varies natural origin and anthropogenic activities. Cr is non-essential and toxic element for plants and is unfavourable to their development and growth. Cr was not detected in ES, OC due to the mostly low uptake of Cr by plants. The mean concentration of Cr determined in Al-Kharj region, Saudi Arabia in the herbal plants is found (0.195 mg/kg – 0.431 mg/kg) which is lower than the presented study (Al-Hammad and Abd El-Salam 2016). (Ali, and Al-Qahtani, 2012).

The mean concentration of Pb in herbal plants samples varied widely from 9.3±0.31 mg/kg – 17.3±0.07 mg/kg, which is higher than the permissible level (2 mg/kg) recommended by WHO. Pb metal is well known as a non-essential toxic for human accumulating in the body, it causes many health problems such as, nervous and immune system problems and learning deficiencies for children, also it is considered as carcinogenic accumulating element (Kananke *et al*., 2014). The Pb toxicities are found in large amounts in many electronic devices and vehicular emissions, which can end up in soil and deposit on the leaves through the atmosphere (Nouri, and Haddioui, 2016). Ali and Al-Qahtani (2012) studied different vegetables gathered from fundamental urban communities in the Kingdom of Saudi Arabia reported that Pb concentrations is (0.54 µg/mg – 6.98 µg/mg) (Ali, and Al-Qahtani, 2012).

The mean concentration of Ni metal in herbal plants varied between 12.6±0.04 mg/kg – 24.4±0.09 mg/kg. The permissible limit of Ni by WHO is 10 mg/kg, all the concentration values were higher than the recommended limit. Ni has been thought to be a fundamental element for human and creature health problems and furthermore retained effectively and quickly by plants (Ogundele *et al.*, 2015). The highest mean concentration of Ni in the herbal plants found for this work is higher contrast with high mean concentration of Ni stated in Al-Kharj region, Saudi Arabia (5.28 mg/kg) (Al-Hammad and Abd El-Salam, 2016). On the other hand, in Morocco reported higher amount of Ni (85 mg/kg) than this work (Al-Jaboobi *et al*., 2014).

Copper (Cu) metal occurs generally in soil, sediments and air is a micro element, which is essential for the plant metabolism and growth. The concentration of Cu in herbal plants ranged between 15.4±0.06 mg/kg to 28.6±0.26 mg/kg (Figure 1), which is higher than the permissible limit according to WHO standard (10 mg/kg). This agrees with the study carried out by (Ali and Al-Qahtani, 2012) in Saudi Arabia as mentioned previously.

Figure 2 shows the contamination factor (CF) in herbal plant samples. The CFs values were 2.54, 1.86, 1.74, 1.78 for Cu, Ni, Cr, Zn respectively, indicating that the herbal plants have a moderate degree of pollution. The value of CF for Pb 8.65 and Fe 7.42 in herbal plant, which are higher than 6 indicate a high degree of pollution. This outcome indicates that high levels of trace metals in herbal plants can lead to human health risk, moreover a study located in Sri Lanka found similar results with green vegetables collected from their local market (Kananke *et al*., 2014). Previous study has revealed that the plants of polluted area had high levels of metals compared with the non-polluted area (Magaji *et al*., 2018).

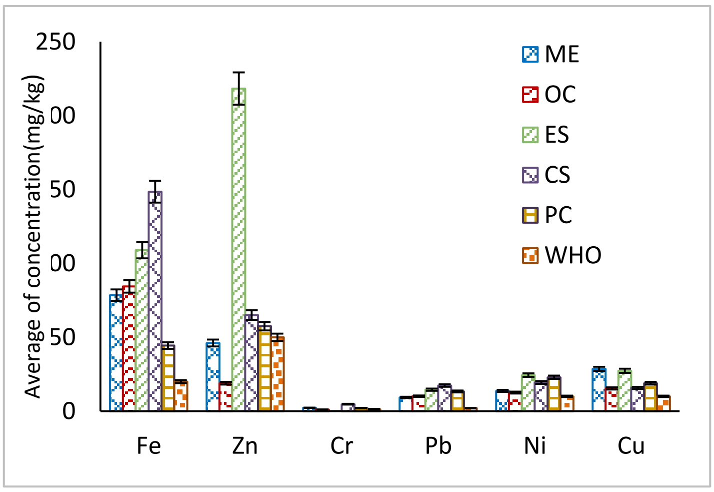
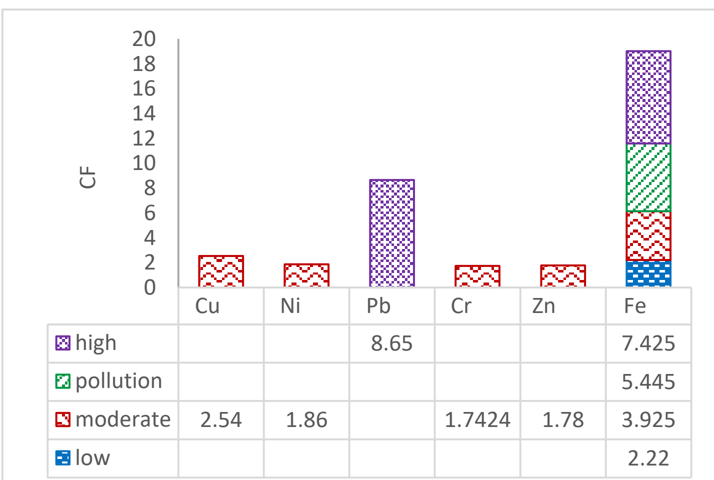


Figure 1. Average concentration of trace metals in herbal plants, WHO



	Cu	Ni	Pb	Cr	Zn	Fe
high			8.65			7.425
pollution						5.445
moderate	2.54	1.86		1.7424	1.78	3.925
low						2.22

Figure 2. The contamination factor (CF) of trace metals in herbal plants

Table 2. One- way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35818.050	5	7163.610	5.309	**0.002**
Within Groups	31036.842	23	1349.428		
Total	66854.892	28			

Table 3. Multiple Comparisons (Latin Square Design LSD) for a significance of trace element

(I) Metal	(J) Metal	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Fe	Zn	11.78000	23.23298	0.617	-36.2811	59.8411
	Cr	90.39000*	24.64229	**0.001**	39.4135	141.3665
	Pb	80.04000*	23.23298	**0.002**	31.9789	128.1011
	Ni	74.34000*	23.23298	**0.004**	26.2789	122.4011
	Cu	71.76000*	23.23298	**0.005**	23.6989	119.8211
Zn	Fe	-11.78000	23.23298	0.617	-59.8411	36.2811
	Cr	78.61000*	24.64229	**0.004**	27.6335	129.5865
	Pb	68.26000*	23.23298	**0.007**	20.1989	116.3211
	Ni	62.56000*	23.23298	**0.013**	14.4989	110.6211
	Cu	59.98000*	23.23298	**0.017**	11.9189	108.0411
Cr	Fe	-90.39000*	24.64229	**0.001**	-141.3665	-39.4135
	Zn	-78.61000*	24.64229	**0.004**	-129.5865	-27.6335
	Pb	-10.35000	24.64229	0.678	-61.3265	40.6265
	Ni	-16.05000	24.64229	0.521	-67.0265	34.9265
	Cu	-18.63000	24.64229	0.457	-69.6065	32.3465
Pb	Fe	-80.04000*	23.23298	**0.002**	-128.1011	-31.9789
	Zn	-68.26000*	23.23298	**0.007**	-116.3211	-20.1989
	Cr	10.35000	24.64229	0.678	-40.6265	61.3265
	Ni	-5.70000	23.23298	0.808	-53.7611	42.3611
	Cu	-8.28000	23.23298	0.725	-56.3411	39.7811
Ni	Fe	-74.34000*	23.23298	**0.004**	-122.4011	-26.2789
	Zn	-62.56000*	23.23298	**0.013**	-110.6211	-14.4989
	Cr	16.05000	24.64229	0.521	-34.9265	67.0265
	Pb	5.70000	23.23298	0.808	-42.3611	53.7611
	Cu	-2.58000	23.23298	0.913	-50.6411	45.4811
Cu	Fe	-71.76000*	23.23298	**0.005**	-119.8211	-23.6989
	Zn	-59.98000*	23.23298	**0.017**	-108.0411	-11.9189
	Cr	18.63000	24.64229	0.457	-32.3465	69.6065
	Pb	8.28000	23.23298	0.725	-39.7811	56.3411
	Ni	2.58000	23.23298	0.913	-45.4811	50.6411

*The mean differences, which are significant at 0.05 level appear in bold.

Trace metals have been found in herbal plants in China, Pakistan, UAE, Saudi Arabia and other Middle East countries (Ernst, 2002; Ulla *et al*., 2012; Begumi *et al*., 2017; Dghaim *et al*., 2015; Maghrabi, 2014; Abou-Arab and Abou Donia, 2000), respectivaly. Based on the result, suggesting that most of the herbal plants suffer from metal pollution might be due to the planting or the way of transportation. Therefore, various factors are contributing to trace metal contamination in herbal plants as a source of agricultural soils pollution, coming from the pesticides, fertilizers, water irrigation, industrial emissions, and atmospheric deposition from town wastes (Mousavi *et al.*, 2014). On the other hand, trace metals contents varied depending on mother country, ecological contamination levels, and processing techniques (Abou-Arab and Abou Donia, 2000; Abu-Darwish, 2009).

The statistical results showed significant difference between group means as determined by one-way ANOVA (**F** (5,23) = 5.309, **p** = 0.002) Table 2. Therefore, to detect the significant difference between them, the Post Hoc Tests was used, and the results are demonstrated in Table 3. These findings suggest that there was no significant difference between elements concentration examined in this investigation ($p > 0.05$). Although, there was a significant difference in the elements appearance in bold with value of ($p < 0.05$) illustrated in Table 3.

4. Conclusions

This examination demonstrated that the herbal plants may represent a significant contributor to the general population as a source of essential elements, such as Fe, Zn, and Ni .On the other hand, some metals such as Cu, Ni, Zn, and Cr are present at level higher than the permissible limits of metals set by WHO. Besides, the herbal plants were found to have a large amount of harmful metals, *e.g.* Pb. Therefore, more consideration should be taken into account to control and check pollution levels in herbal plants. Additionally, from this point considering an action for new study is desired to explore the contamination levels of the trace elements in herbal plants and their correlation with the soil, water irrigation, pesticides, and fertilizers

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