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Determination of Cd, Cu, Pb, Fe and Zn contents in food commodities by using flame atomic absorption spectroscopy in Al-Rass Governorate, Saudi Arabia

Ahmed S. Afify[1,2]*, Adel A. Abdalla[1,3], Bell Gamuhay[1], Abd El-razek Mahmoud[2], Ahmad S. Abu- Khadra[4], M. Hassan[5], M. Ataalla[6] and Amr Mohamed[7,8]

[1]ADECO for Environmental Consultations, 11321-Riyadh, Saudi Arabia.
[2]Department of Applied Science and Technology, Politecnico di Torino, Italy.
[3]Central laboratory of Sohag Company for water and waste water (SCWW/CL), Egypt.
[4]Department of Basic Sciences, Faculty of Engineering Science, Sinai university, Egypt.
[5]Department of Natural Science, Al-Obour Institute of Engineering and Technology, Egypt.
[6]Department of Chemical Engineering, Faculty of Engineering, Badr University in Cairo, Egypt.
[7]Department of Chemistry, Faculty of Science, Taibah University, 41411-Al-Madinah, Saudi. Arabia.
[8]The Higher Institute of Optics Technology (HIOT), Heliopolis, 17361-Cairo, Egypt.

*Corresponding author. E-mail: ahmed.afify@polito.it.

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ABSTRACT

Contamination of food with heavy metals caused by the environmental pollution of air, water and soil is a worldwide public health concern. The concentrations of some heavy metals such as copper, cadmium, lead, iron and zinc in food commodities were determined using wet ashing procedures, followed by flame atomic absorption spectroscopy which allowed the detection of the above mentioned elements present as traces. The food samples were collected from the local markets in Al-Rass Governorate, Al-Qassim region, Saudi Arabia during the period from first of February to end of May 2017. The obtained results of the collected samples showed that the concentrations of the studied heavy metals are within the international standards of the tolerated levels. The study concluded that the highest contents of Cu, Fe, and Zn are in the leafy vegetables. Beside, no potential health hazards on the consumers of these types of food has been reported.

Key words: Atomic absorption spectroscopy, food commodities, heavy metals, wet ashing.

INTRODUCTION

The metallic elements are naturally present in plants, which is essential for normal healthy growth. Conversely, some elements may become of toxic concern when ingested in high doses (Domingo, 1994; Voegborlo, 1993). Some heavy metals, such as lead and cadmium, are the highest toxic among other metallic elements in food, while copper, iron and zinc are naturally present in human diet but may cause health issues to humans under specific circumstances, such accumulation in the kidney and liver which might cause disruption of the biochemical process and many other diseases (Järup, 2003; Moffat and Whittle, 1999). Moreover, food contaminations affect the economics of agricultural industries. Heavy metals have atomic weights ranging from 63.546 to 200.590 (Kennish, 1992) and their specific weight is higher than 4 (Connell and Miller, 1984). The increase in concentrations of those elements may be due to the land disposal of wastes, emission of industrial processes and the contaminations resulting from untreated water or soil (Nollet, 2007). The emission of the compounds of those elements from vehicles represents another important factor that elevates the levels of deposited heavy metals on the surface of vegetables as a result of air pollution; some research on this topic has been conducted in the city of Riyadh, Saudi Arabia (Al Jassir et al., 2005; Al-Rehaili, 2002).

Most of heavy metals are toxic and are not biodegradable, their half-lives are long and they are capable of accumulating in the body due to their solubility in water, thereby causing harmful effects (Nabulo et al., 2011). On the other hand, trace elements play important roles in chemical, biological, biochemical, metabolic, catabolic and enzymatic reaction in the cells of living organism. Cadmium (Cd) is an extremely toxic heavy metal and is identified as a potential hazard with regards to the

quality of edible vegetables. Health agencies have even classified cadmium as a probable human carcinogen. And due to its highly dangerous effect on human health, there is extremely low permissible limit for Cd. Thus a slight exposure to trace amount is already an over exposure. Human intake of cadmium is through food consumption, particularly, vegetables. It is therefore necessary to regulate Cd exposure through vegetable. Cd is concentrated in particular parts of the plants. Plants absorb most of their cadmium from soil through their roots (Arora et al., 2008).

Copper (Cu) is considered a heavy metal due to its atomic number and atomic weight. Industrially, it has numerous uses including in electronics, lighting, electroplating, and others. In a biological context, Cu is an essential trace element that is vital to the health of living beings. It is important for the proper functioning of organs and metabolic processes. The presence of Cu in excess can lead to imbalance in human body. While each mineral has a target organ where it tends to build up, copper can also accumulate in a particular organ and can cause toxicity. Vegetables are particularly high in Cu content and could possibly contribute to copper imbalance in the body (Wilson, 2017).

Iron (Fe) is classified as a heavy metal. It is an abundant element in the earth's crust and industrially, has many uses. Biologically, Fe is an essential part of hemoglobin; the red coloring agent of the blood that transports oxygen through our bodies. However, excessive Fe is also not good for human. Although not most of iron toxicity is caused by free iron, it should be noted that free iron is a pro-oxidant – the opposite of antioxidant – and therefore may cause damage to cells. Hence, absorption of Fe from food must therefore be regulated (Tuso, 2013).

Lead (Pb) is one of the most known toxic elements to the human body and in fact no amount of lead is safe. Eliminating all Pb exposure in our environment is our best course of action. Pb is a heavy metal and often occurs naturally in the soil, which generally ranges from 2 to 60 parts per million (Zhivotovsky et al., 2011). There are many ways that human body can absorb Pb, such as inhalation, ingestion, or dermal contact. Ingestion of Pb can include eating vegetables. Plants absorb Pb from soil through their roots (McBride et al., 2012).

Zinc (Zn)occurs naturally in ores and is abundant in the earth's crust. Zn is an essential mineral and is important for maintaining a healthy immune system, mental alertness, and other functioning of the body organs. While there is no MRL value being set for Zn in vegetable foods, Zn is considered relatively nontoxic. Since Zn is not stored in the body to any practical extent, it is important to obtain enough through diet on a daily basis (Gibson et al., 2014). National and international regulations on food quality set the maximum permissible level of heavy metals in human food (Parliament, 2004; FAO, 1995).

Great attentions have been paid to poisoning caused by metals in food for better investigation. In this regard, large number of samples are needed and the experiment is done in a simple, fast and follows the economic procedure associated with maintaining the level of accuracy and precision (Wieteska et al., 1996). Among the many methods for plant digestion, the dry and wet digestion is a widely used method for the determination of metals in food samples (Buckley and Ihnat, 1993). The wet ashing approach sometimes called wet digestion or wet oxidation is used for the determination of dissolved heavy metals in food. It is used by concentrated nitric acid for destroying the organic materials in tissues of plants, but is favorable to Fe, Cu and Zn due of their content and volatilization during the process of dry ashing (Zarcinas et al., 2008). Combination of different acids and oxidant are mostly implemented in the process of preparation prior to the elemental analysis (Hseu, 2004; Hoenig, 1995; Horwitz, 1970). Many spectroscopic techniques have been used for the determination of heavy metals, such as direct current argon plasma emission spectroscopy (DCP-OES) (Kirkbright and Walton, 1982), Flame atomic absorption spectroscopy (FAAS) (Bacon et al., 2003), graphite furnace atomic spectroscopy (GFAA) (Kebbekus, 2003), inductively coupled argon plasma emission spectroscopy (ICP-OES) (Kebbekus, 2003), and inductively coupled argon plasma mass spectroscopy (ICP-MS) (Dean, 2005). Thus the choice of method depend on the precision, selectivity and sensitivity required.

FAAS is considered as one of the effective solutions for the determination of metals in food owing to its simplicity, economic and easiness of operation.

Due to the severe impact of contamination of food by heavy metals and the sharp increase in chronic disease of Saudi Arabian (WHO, 2016), as well as paucity of reliable data in addition to absence of published reports on vegetables contamination caused by heavy metals in this region, this study was carried out to monitor the concentration of Copper, Cadmium, Lead, Iron and Zinc contents that may be present in common vegetables grown in Al-Rass Governorate to ensure food quality and safety in this region.

MATERIALS AND METHODS

Sample collection

Twenty five types of harvested fresh vegetables and fruits (such as tomato, parsley, green pepper, etc.) were collected from different markets in Al-Rass Governorate, Al-Qassim regions, Saudi Arabia, from the beginning of February 2017 to the end of May 2017. The representative samples were 250 g. The samples were kept in sterile polyethylene bags and transported to the laboratory in an ice chest box. In the laboratory, edible portions of the samples were used for analysis, while bruised or rotten samples were removed. Then samples were cut into small pieces and sliced using a clean knife. They were either

analyzed immediately or stored in polyethylene bags at 4ºC in the fridge until analysis.

Standard solutions/ calibration levels

Copper, Cadmium, Lead, Iron and Zinc stock solutions were of highest purity purchased from Merck, Darmstadt, Germany. 1000 ±0.002 mg/l standard working solutions for each metals of interest was prepared from stock solution immediately before use in mg/l or µg/ml by appropriately diluting with de-ionized water for preparation of calibration standard and spiking experiments.

Drying of samples

All reagents were ACS grade and were purchased from (Panreac, Spain). The Fresh vegetables used in this study were collected from the local vegetable market of Al-Rass Governorate, Al-Qassim region, Saudi Arabia. It has been highly recommended that samples are carefully chosen to represent the original plants and as such, 150-300 g of each vegetable samples were homogenized thoroughly in an electrical grinder (Kenwood CH550, China). Thereafter, 65-70 g of the homogenous sample was weighed in a porcelain crucible using a digital analytical balance (Kern, AE5-4C, Germany). Then it was dried at 105ºC overnight using a drying and heating oven (Binder, ED 53, Germany) to remove the moisture content in the samples. The dried sample was re-weighed to determine the water content (Horwitz, 1970; AOAC, 1994; Marshall, 2010).

Wet ashing of samples

Prior to quantitative analysis by FAAS, it is usually necessary to destroy the organic matrix and bring the element into clear solution. This process can be obtained by wet digested as follows: the dried sample was grinded into a fine powder and 1 g was weighed in a round glass tube using the abovementioned balance.

Afterwards, the sample was dissolved in 5 ml of Nitric acid (69%), which was transferred by single channel micropipette (CAPP, Denmark) and the sample was immersed in a sand path and heated at 120°C using a digital hotplate (Stuart, SD 300,Bibby Scientific Ltd, UK) overnight. Then, 5 ml of Nitric acid 69%, which was added to stop the brownish fumes, and dense white fumes were observed. Some drops of hydrogen peroxide (30% w/v) were added to the solution until it became Clear. Further, the interior wall of the glass tube was washed and the de-ionized water swirled to avoid the loss of the sample. The solution was then allowed to cool down to room temperature and de-ionized water was added to dissolve the precipitate formed on cooling. Thus, the digested sample was filtered in 50 mL in a glass volumetric flask (Duran, class A with PE stopper) using a qualitative filter paper (601:2.5µm - medium speed, Ahlstrom, USA) which is commonly used in the food industry to separate foodstuff in order to avoid the intrusion of particles into the solution. The filtered solution was completed to the mark with de-ionized water using Purelab Flex, ELGA, Veoila water solution and technologies, UK with specific resistivity of 18 mΩ. It was then it mixed properly prior to the elemental analysis (Marshall, 2010). The digested samples were kept in refrigerator until FAAS analysis. All glassware was rinsed with de-ionized water before use and cleaned using heated digital ultrasonic bath (Clifton, DU-14, Nickel, Electro Ltd, Britain) at 40ºC for 10 min.

Heavy metals analysis by FAAS

Samples were analyzed using flame atomic absorption spectroscopy (iCE 3500 AA system, P.N. 942350023500, S.N. AA05140808, Thermo Scientific, UK) with impact beads nebulizer re-quipped with a deuterium background corrector and using hollow cathode lamb for each element of (Cu, Cd, Zn, Fe and Pb) S.N. (15521625, 16301561, 16290581, 16261015 and 13520036), respectively at specific wave length of every element. For each element, respective hollow cathode lamp was inserted in to the FAAS and the solution was successively aspirated into the flame. The flow rate of acetylene and air was managed to ensure appropriate flame conditions. Analysis was performed in three replicate for each sample using absorption/concentration mode; the nebulizer uptake time was 4 s and normal segmented curve method was used (Figure 1).

Data acquisition

SOLAAR AA Software, (SOLAAR Data Station, v11.03, Thermo Fisher Scientific Inc.) was used for data acquisition. The results are means of three replicates. Measurements were done against metals standard solutions. The heavy metal levels were based on plants dry weight. The results were expressed as mg metal per kg. The working conditions are described in Table 1.

RESULTS AND DISCUSSION

Quality control

To check the quality and usefulness of the optimized method for the determination of Copper, Cadmium, Lead, Iron and Zinc contents in food, appropriate procedures and precautions were carried out to ensure the reliability of the obtained results. Table 2 shows the concentration of the working solutions standard and the value of

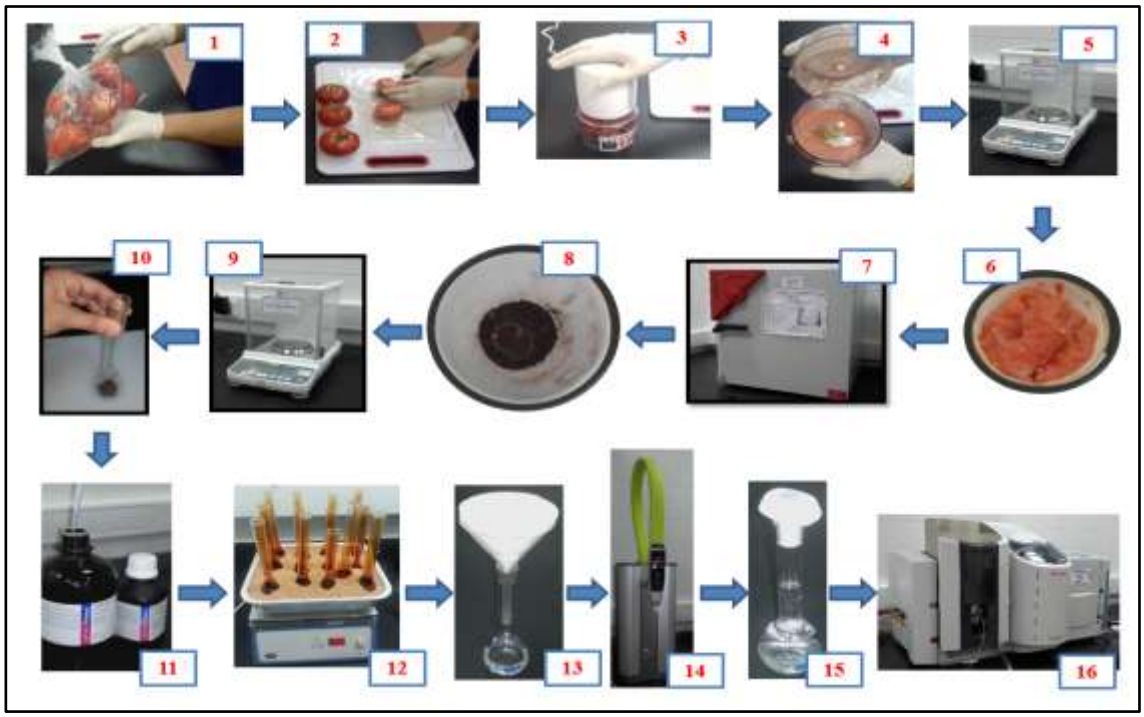

Figure 1: Schematic diagram of the determination of heavy metals in tomato using FASS.

Table 1: The working conditions of FAAS.

Item	**Heavy Metals Under Investigation**				
Element	**Cu**	**Cd**	**Zn**	**Fe**	**Pb**
Flame type	Air/acetylene				
Fuel flow	1.1	1.1	1.2	0.9	1.1
Wavelength (nm)	324.8	228.8	213.9	248.3	217
Calibration levels	0.25, 0.50, 1.00, 2.00	0.05, 0.10, 0.20, 0.40	0.125, 0.250, 0.500, 1.0	0.50, 1.00, 2.00, 4.00	0.05, 0.10, 0.20, 0.40
Lamp current (mA)	5	8	10	15	10
Signal	3.5 mg/0.4 A	1.5 mg/0.4 A	1 mg/0.4 A	5 mg/0.4 A	7 mg/0.4 A
Bandpass(width) (nm)	0.5	0.5	0.2	0.2	0.5

Table 2: Thequality control parameters of the elemental analysis.

S/N	**Laboratory sample control (LCS)_ ID**	**Heavy metals under Investigation**				
		Fe	**Cu**	**Zn**	**Pb**	**Cd**
1	0.25 (ppm)	0.27	0.246	0.27	0.053/0.05	0.040 /0.05
2	0.5 (ppm)	0.53	0.506	0.52	NA	NA
3	REC.%	106-108%	98.4–104%	104-108%	106%	80 %
4	LOD	0.0043	0.0045	0.0033	0.013	0.0028
5	LOQ	0.013	0.014	0.0103	0.04	0.0088
6	Correlation Coefficient	0.999	0.9999	0.996	0.999	0.996

correlation coefficient for each metal.

Linearity

The range of linearity of concentration using standard solution in mg/l or µg/ml for the fifth elements versus absorbance graph is important for the determination of element concentration in food samples and the observed correlation coefficient mentioned in the Table 3. Although for Fe and Cu measurements, a dilution of digested solution with deionized water is required.

Table 3: Distribution of concentration Cu, Cd, Pb, Fe and Zn in ppm in food commodities.

S.N.	Sample type	No. of samples	Cd	Pb	Cu	Zn	Fe
			(ppm)				
	Beans	3					
1	Average		<LOQ	<LOQ	<LOQ	0.08	0.19
	Range		<LOQ	<LOQ	0.0 - 0.01	0.06 – 0.12	0.14 – 0.30
	Broccoli	2					
2	Average		<LOQ	<LOQ	<LOQ	0.10	1.33
	Range		<LOQ	<LOQ	<LOQ	0.05 - 0.15	1.32 - 1.33
	Cabbage	6					
3	Average		<LOQ	<LOQ	<LOQ	0.02	0.06
	Range		<LOQ	<LOQ	0.0 - 0.01	0.01 - 0.03	0.02 - 0.09
	Carrot	7					
4	Average		<LOQ	<LOQ	<LOQ	0.03	0.19
	Range		<LOQ	<LOQ	0.0 - 0.01	0.02 - 0.06	0.04 - 0.77
	Coriander	16					
5	Average		<LOQ	<LOQ	0.01	0.05	0.95
	Range		<LOQ	<LOQ	0.01 - 0.03	0.02 - 0.09	0.03 - 3.18
	Cucumber	24					
6	Average		<LOQ	<LOQ	<LOQ	0.04	0.05
	Range		<LOQ	<LOQ	0.01 – 0.03	0.01 – 0.07	0.0 – 0.15
	Dill	5					
7	Average		<LOQ	<LOQ	<LOQ	0.05	1.21
	Range		<LOQ	<LOQ	<LOQ	0.03-0.07	0.27-4.04
	Eggplant	20					
8	Average		<LOQ	<LOQ	<LOQ	0.04	0.05
	Range		<LOQ	<LOQ	0.00-0.03	0.01-0.08	0.00-0.15
	Green chili pepper	17					
9	Average		<LOQ	<LOQ	0.01	0.05	0.12
	Range		<LOQ	<LOQ	0.01 – 0.05	0.01 – 0.17	0.04 – 0.24
	Green onion	7					
10	Average		<LOQ	<LOQ	<LOQ	0.07	0.18
	Range		<LOQ	<LOQ	0.0 – 0.01	0.01 – 0.33	0.01 – 0.39
	Green pepper	20					
11	average		<LOQ	<LOQ	<LOQ	0.03	0.09
	Range		<LOQ	<LOQ	0.0 – 0.02	0.02 – 0.04	0.05 – 0.18
	Leek	13					
12	Average		<LOQ	<LOQ	0.01	0.08	1.06
	Range		<LOQ	<LOQ	0.01 – 0.04	0.02 – 0.53	0.28 –2.83
	Lemon	2					
13	Average		<LOQ	<LOQ	<LOQ	0.01	0.12
	Range		<LOQ	<LOQ	<LOQ	0.00 – 0.01	0.05 – 0.18
	Lettuce	13					
14	Average		<LOQ	<LOQ	<LOQ	0.04	0.57
	Range		<LOQ	<LOQ	0.00 – 0.01	0.01 – 0.08	0.03 – 1.42
	Mint	7					
15	Average		<LOQ	<LOQ	0.02	0.05	1.96
	Range		<LOQ	<LOQ	0.01 – 0.03	0.01 – 0.09	0.05 – 5.24

Table 3: Conts. Distribution of concentration Cu, Cd, Pb, Fe and Zn in ppm in food commodities.

	Okra	5					
16	Average		<LOQ	<LOQ	0.03	0.10	0.20
	Range		<LOQ	<LOQ	0.02 – 0.04	0.08 – 0.18	0.07 – 0.29
	Parsley	22					
17	Average		<LOQ	<LOQ	0.02	0.08	1.35
	Range		<LOQ	<LOQ	0.01 – 0.07	0.01 – 0.48	0.07 – 4.89
	Potato	4					
18	Average		<LOQ	<LOQ	0.01	0.04	0.23
	Range		<LOQ	<LOQ	0.00 – 0.01	0.03 – 0.05	0.15 – 0.26
	Pumpkin	3					
19	Average		<LOQ	<LOQ	0.02	0.03	0.15
	Range		<LOQ	<LOQ	0.00 – 0.03	0.03 – 0.04	0.10 – 0.25
	Radish	2					
20	Average		<LOQ	<LOQ	<LOQ	0.03	0.14
	Range		<LOQ	<LOQ	<LOQ	0.00 – 0.03	0.05 – 0.23
	Spinach	4					
21	Average		<LOQ	<LOQ	0.02	0.04	0.49
	Range		<LOQ	<LOQ	0.00 – 0.03	0.02 – 0.06	0.13 – 1.05
	Squash	27					
22	Average		<LOQ	<LOQ	0.01	0.06	0.21
	Range		<LOQ	<LOQ	0.00 – 0.04	0.02 – 0.14	0.00 – 1.18
	String beans	3					
23	Average		<LOQ	<LOQ	0.01	0.08	0.16
	Range		<LOQ	<LOQ	0.00 – 0.01	0.06 – 0.10	0.10 – 0.23
	Tomato	42					
24	Average		<LOQ	<LOQ	<LOQ	0.02	0.29
	Range		<LOQ	<LOQ	0.00 – 0.02	0.00 – 0.06	0.00 – 0.38
	Watercress	12					
25	Average		<LOQ	<LOQ	<LOQ	0.04	0.93
	Range		<LOQ	<LOQ	0.00 – 0.02	0.02 – 0.06	0.13 – 3.13

*Samples that were considered to be non-detections were assigned a lower than limit of quantification (<LOQ).

Detection limit

It is defined as the concentration corresponding to three times the standard deviation of ten blanks as shown in Table 2.

Recovery

It was carried out to check the accuracy of the method, where an adequate amount of the metal standards were added to the selected sample by spiking and then reanalyzing the samples. The percentage of recovery can be seen in the Table 2. It is worth mentioning that the percent recovery should not be less than 75% or greater than 125% (USEPA, 2007).

Elemental analysis by FAAS

Prior to the analysis, the nebulizer was cleaned by aspirating with 5 mL of de-ionized water. Thereafter, the blank solution was prepared following the same digestion procedures, and standard solutions were aspirated first and followed by unknown food samples for each measurement.

The results of the present study for the concentration of copper, cadmium, lead, iron and zinc contents in different food commodities collected from Al-Rass Governorate, Saudi Arabia are given in Table 3. The average (mean) and the range of each element for each sample are given in the last two rows.

The obtained result of this study shows that the concentration of Cd and Pb are either negligible or

undetectable in all investigated samples. The main cause of contamination of crops with these two elements can be ascribed to the heavy traffic near to the cultivated lands or markets, which is not the case in Al-Rass area, as it is very quiet as compared with previous studies performed in other big cities (Ali and Al-Qahtani, 2012).

The highest Cu content was observed in the leafy vegetables, such as parsley and mint, ranging from 0.01 up to 0.07 ppm which is in a good agreement with other literature (Ali and Al-Qahtani, 2012), where the values of Cu was almost undetectable in some types such as broccoli, lemon and radish.

The detected levels of Zn shows that leek, parsley and green onion have the highest concentrations of 0.08, 0.07and 0.07 ppm respectively, where the lowest values of Zn were 0.01, 0.02, 0.02 ppm for lemon, cabbage and tomato, respectively.

Iron was the most abundant heavy metal in all investigated samples. The concentration of Fe was much higher in leafy vegetables than other vegetables as the leaves are considered the food making factories in leafy plants which, in turn, increases the Fe uptake in their leaves. Mint, parsley, dill and rocket showed the highest content of Fe at 1.96, 1.35, 1.21 and 0.93 ppm respectively, which is in a good agreement with other literature (Demirezen and Aksoy, 2006). The lowest values of Fe were for cucumber, eggplant and cabbage at 0.05, 0.05 and 0.06 ppm, respectively. The high content of heavy metals in leafy vegetables as compared with other vegetables is due to the act of leaves as a comparatively large entry points of the deposits of the compounds of such elements from air (Demirezen and Aksoy, 2006).

Conclusion

A simple, yet efficient, digestion method was developed for the analysis of heavy metals in some food commodities cultivated and/or marketed in Al-Rass Governorate, Al-Qassim region, Saudi Arabia. The level of five heavy metals was determined using FAAS. Copper, zinc and iron are commonly found in the region although iron was the most abundant heavy metal in this region, while Cd and Pb were undetectable in any of the agricultural food commodities. The present study demonstrated that the concentrations of heavy metals in all types of vegetables are within the limits of global standards. Therefore, further studies should be conducted to monitor the bioaccumulation of the detected heavy metals in order to assess their health risks upon exposure in the investigated area.

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