RESEARCH ARTICLE

Evaluating of Heavy Metal Contaminations in the Most Applicable Food Spices and Flavors in Hamedan, Iran

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Background & Aims of the Study: Iranian food diet contains nutrients and herbal spices. From the most important challenges in relation to these spices is their pollution with various heavy metals. Thus, this study aims to characterize the amounts of heavy metals in collected spices from Hamedan city of Iran during 2015-2016.

Materials & Methods: 180 samples of commercially accessible vegetal spices were collected from local stores all over the Hamedan city. The samples were weighted and dried in an electrical oven at 105 ±1 °C for 24 h. Then, samples were grounded to powder by a grinder. Afterwards, the samples were digested by adding 4 ml and 1 ml of concentrated nitric acid and hydrogen peroxide, respectively. After filtration of samples, flamed atomic absorption used for detection of Zinc, Copper, Iron, Nickel, Manganese, Cadmium and Lead concentrations. For the statistical analysis of the results, SPSS version 21 was used. For examining data normality, Kolmogorov-Smirnov test; to compare the mean concentration of elements between spice samples from Tukey test and in order to investigate the correlation between the average concentrations of the heavy metals in the samples, Pearson correlation test were used. Comparing mean concentration of elements with standard values was conducted, using one-sample t-test.

Results: The mean concentrations of Cadmium as a toxic trace element were low in all samples and no risk threatens consumers. But, lead content in dried mint (6.04±0.85mg/kg) was high compared to the standard values. This trend was followed by cinnamon (4.88±1.32), turmeric (2.05±0.63), black pepper (1.51±0.63), sumac (1.17±1.08) and red pepper (0.72±0.85).

Conclusion: Lead in dried mint exceeded the standard value whose reason can be the increase of population, mining, industrialization, transportation and usage of chemical fertilizers, leading to the spread of environmental pollution. Thus, more serious health cares and regular periodic supervisions on food spices regarding pollution with this element seem necessary.

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Background

Nowadays, herbal medicines are commercially used in many countries for the reasons such as growing general interest in natural treatments (1,2). Use of different herbal compounds and products has increased in recent years for their useful effects on human health, relative low price and the knowledge deficiency of people about their likely harmful effects (3). Among the most applicable and favorite herbal products, spices can be mentioned as the inseparable components of daily life in various societies. Various vegetal spices are widely applied in human diet all over the world (4). The most famous of them are including turmeric, cinnamon, red pepper, black pepper, sumac and dried mint. Besides using of these spices as the flavors for making color and odor

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in the food, they are also used for their enormous benefits for the human health (5). For example, turmeric has anti-cancer effects, removes digestion disorders, ear infections, repairs skin damages; cinnamon regulates blood sugar and overweight; red pepper has anti-pain properties, treats diabetes, repairs dermal hurts; black pepper is anti-constipation, removes abscess, toothache, different sunburns and eye problems; sumac blocks bleeding, diarrhea, treats ear infections and eye trachoma, inhibits chickenpox and decreases blood cholesterol; dried mint treats indigestion, decreases blood cholesterol, improves nervous system, soothes earache, opens respiratory system and decreases allergic symptoms (6-8). General attitude among people is that since herbal medicines have natural origins are without danger and the lack of side effects on the consumers. However, since using cultivated herbal medicines in the polluted areas or with improper processing can be one of the entrance ways of dangerous pollutants such as heavy metals to the humans and animals´ bodies, controlling them regarding the presence of heavy metals seems necessary (9,10). Simultaneous with growing improvements in human life in different sections such as agriculture, industry, transportation and mines, different pollutants like heavy metals have entered the environment, finally penetrating into the food chain and the bodies of consumers (11,12). Heavy metals are a group of mineral pollutants that have occupied a considerable part of environmental pollution (13,14). Regarding biological accumulation capability and long-term consistency in the environment, these elements are toxic for the living organisms even in low concentrations and not decomposed in their bodies (15,16). Metabolism and removing these toxic elements in the body and their absorption is slower, leading to the toxicity, diseases and even death of living organisms depending on their entered values (17,18). One entrance way of these toxic elements to human body is through using herbs that have grown in

the contaminated soils (15,17). Since spices and different herbal flavors are frequently and daily used in the diets, determining the level of these elements is significantly important; because they can have a direct impact on human body health and in case of imbalance of their values in food materials, the food can turn into a harmful factor for human body (8).

Aims of the study:

Since no study has been conducted on measuring concentration level of heavy metals in food spices of Hamedan city, this research aimed to detect the concentrations of various elements (Zinc, Copper, Iron, Nickel, Manganese, Cadmium, and Lead) in the mostly-consumed spices and flavors (cinnamon, turmeric, black pepper, sumac, red pepper and dried mint) collected in Hamedan city during 2015-2016 and comparing them with global standards.

Materials & Methods

Sample collection

180 samples of highly used spices containing 10 famous commercial brands with three replications including cinnamon (n=30), turmeric (n=30), black pepper (n=30), sumac (n=30), red pepper (n=30) and dried mint (n=30) were purchased randomly from the stores from different regions of Hamedan city in the period 2015-2016. They were transferred to the Food Hygiene and Quality Control lab of Veterinary Science Faculty of Bu-Ali Sina University for examination. Names and features of used samples are represented in Table 1.

All used containers and glasses were immersed in 0.1Normal nitric acid for 24h. Then, all containers were rinsed with distilled water and dried in electrical oven.

Sample preparation

In the lab, 1g of the samples were weighted and put in an electrical oven (Labinco) at 105˚C for 24h. Then, the samples were ground and 0.5g of the obtained powder was transferred to the digestion pipe. Afterwards, 4ml of 68% nitric

acid and 1ml of 30% hydrogen peroxide were poured on the obtained sample.

Table 1) Characteristics of tested samples

English name	Scientific name	Family	Used component
Turmeric	*Curcuma longa*	Zingiberaceae	root
Cinnamon	*Cinnamomum verum*	Lauraceae	bark
Red Chili	*Capsicum annuum*	Solanaceae	fruit
Black pepper	*Piper nigrum*	Piperaceae	fruit
Sumac	*Rhus coriaria*	Anacardiaceae	fruit
Dried mint	*Mentha spicata*	Lamiaceae	leaf

Digestion process was conducted on an electronic heater (Kern) at 130 ˚C and under a chemical ventilator to gain a translucent solution. After digestion, samples were cooled and for removing any suspending materials they were filtered by a filtration paper Watman No.42. Then, the volume of obtained solution was adjusted to 10 ml by adding of twice distilled water (5).

Measurement of heavy metals

In order to detect the concentration of zinc, copper, iron, nickel, manganese, cadmium and lead in the samples, first, mother and standard solutions of salt were determined for each element; after calibrating flamed atomic absorption device of Varian (model AA-220), the concentration of the elements were read. All features of flamed atomic absorption device are represented in Table 2. For making sure about the correctness of the results, absorption values of unknown samples were replicated for three times (5).

Table 2) Characteristics of flamed atomic absorption

Elements	Wave length	(nm) Split width	(mA) Lamp current	Detection range (mg/kg)	Standard solution (mg/kg)		
Zn	213.9	1	5	0.01-3	1	2	3
Cu	324.7	0.5	4	0.02-3	1	2	3
Fe	248.3	0.2	5	0.06-15	2	5	10
Ni	232	0.2	4	0.01-20	1.5	3	6
Mn	279.5	0.2	5	0.02-5	1	3	5
Cd	228.8	0.5	4	0.02-3	1	2	3
Pb	217	1	5	0.1-30	2.5	5	10
Flame type	Acetylene-air						
Read time (s)	3						
Lamp type	HCL						
Measurement type	Absorbance						

Data analysis:

For the statistical analysis of the results, SPSS version 21 was used. For examining data normality, Kolmogorov-Smirnov test; to compare the mean concentration of elements between spice samples from Tukey test and in order to investigate the correlation between the average concentrations of the heavy metals in the spices, Pearson correlation test were used. Comparing mean concentration of elements with standard values was conducted, using one-sample t-test.

Results

Mean concentrations of the elements (zinc, copper, iron, nickel, manganese, cadmium, and lead), based on mg/kg in turmeric, cinnamon, red pepper, black pepper, sumac and dried mint are represented in Table 3. Correlation of mean accumulated values of heavy metals in the collected samples is shown in Table 4.

Results of normality of the elements´ concentrations in the study samples showed that concentration of all elements has normal distribution. Table 4 shows the correlation coefficient of different elements. Thus, a significant positive correlation at the level (p<0.05) and correlation coefficient of r >0.5 was observed for iron-copper (r=0.626), nickel-

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copper (r=0.600), nickel-iron (r=0.915), manganese-iron (r=0.607), manganese-nickel (r=0.517), cadmium- manganese (r=0.505) and lead- cadmium (r=0.701). Based on Table 5, values of zinc, copper, iron, nickel, and manganese in all samples were significantly (p<0.05) different with standard values and are lower than standard limits. In case of cadmium, only dried mint was not significantly different

with WHO guidelines. Whereas, the rest of the samples significantly (p<0.05) were lower than the standard value. The values of lead in turmeric, cinnamon, red pepper, black pepper, and sumac were significantly (p<0.05) different and lower than WHO guidelines. But, the value of lead in the dried mint was significantly higher than the standard limit.

Table 3) Mean ± Standard deviation of heavy metal concentrations in studied samples

					(mg/kg) Studied elements		
Samples	**Zn**	**Cu**	**Fe**	**Ni**	**Mn**	**Cd**	**Pb**
Turmeric	4.90±1.41[a]	2.49±0.85[a]	55.89±8.57[a]	0.33±0.72[a]	70.38±6.66[c]	0.06±0.071[a]	2.05±0.63[b]
Cinnamon	5.46±2.04[a]	3.31±0.85[a]	98.34±9.16[b]	0.20±0.11[a]	25.63±8.42[b]	0.19±0.08[b]	4.88±1.32[c]
Red pepper	9.53±1.61[b]	8.38±1.97[c]	56.79±6.28[a]	0.35±0.81[a]	25.48±٤/٥٩[b]	0.13±0.09[b]	0.72±0.85[a]
Black pepper	11.40±3.24[b]	9.92±1.61[c]	222.33±5.20[e]	1.35±1.60[b]	25.10±8.07[b]	0.15±0.06[b]	1.51±0.63[ab]
Sumac	10.21±4.05[b]	3.47±0.87[a]	152.75±9.19[c]	0.38±0.73[a]	9.15±2.60[a]	0.14±0.06[b]	1.17±1.08[ab]
Dried mint	19.62±3.55[c]	6.35±1.67[b]	170.49±10.54[d]	0.09±0.23[a]	70.71±14.41[c]	0.34±0.07[c]	6.04±0.85[c]

Different letters in each column indicate a statistically significant difference (P≤0.05).

Table 4) Correlation relationship between the studied element concentrations

	Zn	**Cu**	**Fe**	**Ni**	**Mn**	**Cd**	**Pb**
Zn	1.000						
Cu	0.361	1.000					
Fe	0.147	0.626[*]	1.000				
Ni	0.103	0.600[*]	0.915[*]	1.000			
Mn	-0.185	00.20	0.607[*]	0.517[*]	1.000		
Cd	0.055	-0.125	-0.180	-0.260	0.505[*]	1.000	
Pb	0.387	-0.198	-0.211	-0.269	0.263	0.701[*]	1.000

Discussion

Comparison of reported values in this study to other studies conducted across the world and to the global standards is represented in Table 5. Generally zinc, copper, iron, nickel, and manganese are classified as minor elements whose concentration is higher than 1mg/kg in food on average (19). According to the results, the mean concentrations of all minor elements (Zinc, Copper, Iron, Nickel and Manganese) in the all spices were in the proper nutritional range, based on FAO/WHO standards.

Zinc

Zinc is found in about every cell of the body, playing a vital role in its development. Zinc has a determining role in the performance of over 300 enzymes in the body, participating in their building or regulating their activities. But, high values of zinc in the body interfere with the metabolism and activities of other metals (9).

Based on Table 3, maximum value of zinc with the concentration of 19.62±3.55 mg/kg belonged to the dried mint, black pepper (11.4±3.24), sumac (10.21±4.05), red pepper (9.35±1.61), cinnamon (5.46±2.04) and turmeric (4.90±1.41) obtained the next ranks respectively. Obtained zinc values in all spices were significantly lesser than the standard value suggested by WHO (60 mg/kg) in present study. In agreement with this finding, previous studies, all reported zinc values below 60 mg/kg in turmeric, cinnamon, red pepper, black pepper, sumac, and dried mint (8,17,19-22). While, Singh et al. (2006) reported zinc value of turmeric, cinnamon, and red pepper higher and about black pepper lower than standard limit (23). Zinc value of these herbs depends on the existing zinc on the surface of their culture, weather conditions, geographic location, cultivation method, and its measurement method in the samples (19).

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Table5) Comparison of the obtained concentrations in present study with recent studies and Maximum Permissible Limits

Sample	Present study	Zn	Cu	Fe	Ni	Mn	Cd	Pb
		Elements (mg/kg)						
Turmeric	(8)	4.90±1.41	2.49±0.85	55.89±8.57	0.33±0.72	70.38±6.66	0.06±0.071	2.05±0.63
	(19)	26	23.8	210	-	26.8	0.012	3.9
	(23)	30.90	3.08	-	1.14	59.63	0.04	0.121
	(20)	101	0.12	45	-	-	-	-
	(21)	16.5	2.90	71	0.36	14.2	0.008	-
Cinnamon	Present study	18.3	5.3	800	-	16.5	-	-
	(17)	5.46±2.04	3.31±0.85	98.34±9.16	0.20±0.11	25.63±8.42	0.19±0.08	4.88±1.32
	(8)	12.4	4.7	158.9	0.77	159.7	0.14	0.76
	(19)	21	6	78	-	58	0.18	11.7
	(23)	13.75	2.83	-	1.59	879.8	0.036	0.162
	(20)	104	-	2390	-	83	-	-
	(22)	38.3	2.78	53	0.32	156	0.118	-
	(21)	13	4.68	108	0.72	264	-	0.310
Red pepper	Present study	10	4	129	-	323	-	-
	(17)	9.53±1.61	8.38±1.97	56.79±6.28	0.35±0.81	25.48±4.59	0.13±0.09	0.72±0.85
	(23)	7.84	4.2	234.1	2.92	11.9	0.65	0.79
	(20)	105	-	287	-	-	-	-
	(21)	32.7	11.7	339	6.5	17.6	0.039	-
Black pepper	Present study	22.8	141	3708	-	194	-	-
	(17)	11.40±3.24	9.92±1.61	222.33±5.20	1.35±1.60	25.10±8.07	0.15±0.06	1.51±0.63
	(8)	15.5	9.4	281.8	4.99	14.9	0.93	1.44
	(23)	29.4	19.2	390	-	52	0.046	3.8
	(20)	37.1	-	76	-	73.3	-	-
	(21)	22	10.19	158	4.04	164.7	-	-
Sumac	Present study	5	14.3	155	-	237	-	-
	(17)	10.21±4.05	3.47±0.87	152.75±9.19	0.38±0.73	9.15±2.60	0.14±0.06	1.17±1.08
	(8)	19.9	28.7	247.7	3.64	13.4	0.11	0.47
Dried mint	Present study	20.5	44	130	-	26	0.045	5.5
	(17)	19.62±3.55	6.35±1.67	170.49±10.54	0.09±0.23	70.71±14.41	0.34±0.07	6.04±0.85
	(8)	21.7	9.7	376.3	8.69	78.9	0.5	1.14
	(20)	36	16	650	-	45	0.05	6.6
	(21)	23.6	10.04	281	2.40	98	0.02	-
	MPL (mg/kg)*	40.3	30.6	4144	-	92.5	-	-
Standard value		60	40	300	1.63	100	0.3	5

*MPL = Maximum Permissible Limits (25-27).

Copper

Copper exists in the structure of proteins and necessary metal enzymes for body metabolism. It plays an important role in bone growth, connective tissue, brain, heart and many other organs. Copper is a necessary factor in making red globules, absorption and use of iron, cholesterol and glucose metabolism (9,19). Based on the results, copper values in the studied samples are as follows: black pepper (9.92±1.61), red pepper (8.38±1.97), dried mint (6.35±1.67), sumac (3.47±0.87), cinnamon (3.31±0.85) and turmeric (2.49±0.85), respectively. Copper concentration of all studied samples was significantly ($P<0.05$) lower than standard value (40 mg/kg) suggested by WHO in present study. Other researchers also found copper value of turmeric, cinnamon, red pepper, black pepper, sumac, and dried mint lower than 40 mg/kg (8,17,19-23). But, Ibrahim et al. (2012) reported copper value higher than the standard limit in sumac samples (8).

Iron

Iron is a vital element in the animals and herbs. This element in the herbs is mostly found in the form of Phytat complexes and non-organic iron salts such as ferric hydroxide (9). Iron is the most important element for producing blood cells in the body in a way that 70% of iron is

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inside blood cells and in the form of hemoglobin with a vital role in transferring oxygen to the human tissues (19). Based on Table 3, maximum iron value was observed in the black pepper (222.33±5.20), followed by dried mint (170.49±10.54), sumac (152.75±9.19), cinnamon (98.34±9.16), red pepper (56.79±6.48), and turmeric (55.89±8.75), respectively. Comparing these results with the standard limit of 300 mg/kg suggested by WHO showed that found iron values in all samples were significantly lower (p<0.05). Results of previous studies on iron in various spices were very different. Disagreed with this study, Soylak et al. (2004) reported iron in dried mint, Ibrahim et al. (2004) examined iron in dried mint and black pepper; Singh et al. (2006) showed iron in cinnamon; Karadas and Kara (2012) reported iron in red pepper; Ansari et al. (2004) examined iron in cinnamon, red pepper and dried mint higher than maximum permissible limits (300 mg/kg) (8,17,20,21,23). Except for the mentioned studies, reported iron values in the studied spices by previous studies were all in the standard range (Table 5).

Nickel

According to Table 3, maximum nickel value in the samples related to black pepper (1.35±1.60), followed by the sumac (0.38±0.73), red pepper (0.35±0.81), turmeric (0.33±0.72), cinnamon (0.2±0.11) and dried mint (0.09±0.23), respectively. Found nickel value in all samples was significantly (p<0.05) lesser than the maximum permissible limits (1.63 mg/kg) suggested by WHO (20). Another study found higher than standard nickel value in sumac, red pepper and black pepper besides dried mint (17). Except for the mentioned items, results of other reports about nickel value of the studied spices by previous researchers agreed with the results of this study and all were found to be under 1.63 mg/kg (Table 5). Nickel was a necessary element for iron absorption, preventing anemia in the body. This element attends in making enzymes such as

dehydrogenases, transaminases, and alpha amylases that handle many chemical reactions of the body. Also, nickel plays a main role in absorbing calcium in the bones (19).

Manganese

Among studied samples, manganese value in the dried mint (70.71±14.41 mg/kg) was the highest, followed by turmeric (70.38±6.66), cinnamon (25.63±8.42), red pepper (25.48±4.59), black pepper (25.10±8.07) and sumac (9.15±2.60), respectively. Obtained manganese values in all samples were significantly (p<0.05) lower than given standard value (100 mg/kg). Despite the results of this study, reported manganese value of some researchers in cinnamon was higher than 100 mg/kg (17,19,22). Besides cinnamon, Karadas and Kara (2012) found high value of manganese in black pepper (20). Another study reported higher than 100 mg/kg manganese values in red pepper (21). Except for mentioned studies, manganese value in the studied spices by other researchers was found to be in the standard range (Table 5). Manganese in the body helps building connective tissues, bones, bleeding clotting factors and sex hormones. Manganese has a vital role in the metabolism of carbohydrate, fat, calcium absorption, and regulating blood sugar. Also, manganese is vital for the natural performance of brain and nerves (19).

Since the concentrations of zinc, copper, iron, nickel, and manganese in the collected spices didn't exceed the standard value of WHO, it can be concluded that these spices are good resources for providing these elements in the body.

Another classification of heavy metals is trace elements divided into two groups of toxic trace elements and nontoxic trace elements. On average, the value of this taxonomy is lower than 1 mg/kg in the food. Two elements of cadmium and lead are grouped in toxic trace elements by WHO since they are toxic for humans and other mammals (19). So, they are

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main challenges in food that should be probed in food materials.

Cadmium

Researchers have attributed cadmium increase in the herbs to the use of cadmium-containing fertilizers and watering plants with swage (8). Its high value in the body induces kidney injuries, leading to aminoaciduria, glycosuria and renal tubule necrosis (9). In this study, maximum reported cadmium value (mg/kg) belonged to dried mint (0.34±0.07), cinnamon (0.19±0.08), black pepper (0.15±0.06), sumac (0.14±0.06), red pepper (0.13±0.09) and turmeric (0.06±0.71), respectively. Except for dried mint, all obtained values were significantly lower than the standard value (0.3 mg/kg). Cadmium value of dried mint was not significantly different with the reported standard value. In one study, cadmium value in the red pepper, black pepper and dried mint was reported to be higher than 0.3 mg/kg (17). While, similar to this study, previous reports about studied spices contained lower than standard values of cadmium (table 5).

Lead

Lead is a dangerous toxic metal, leading to the disorders in hemoglobin making and Porphyrin metabolism by bounding to the enzymes. Its accumulation in the human body leads to the brain injuries and mental retardation (8,24). Maximum lead value in this study related to the dried mint with the concentration of 6.04±0.85, cinnamon (4.88±1.32), turmeric (2.05±0.63), black pepper (1.51±0.63), sumac (1.17±1.08) and red pepper (0.72±0.85 mg/kg), respectively. Lead value in the dried mint was significantly higher than maximum permissible limits ($p < 0.05$); thus, it can be considered a warning for the consumers. In one study, lead value in the dried mint, cinnamon and sumac was higher than the standard value (8). But, lead value in the studied spices was lower than the standard value in other studies (Table 5). High concentration of cadmium and lead in the dried mint can be attributed to growing air pollution, lead penetration during grinding

spices in the factory, use of various pesticides, different agricultural fertilizers, increased industrialization of communities, presence of mines etc.

Conclusion

According to the our findings, the values of zinc, copper, iron, manganese and nickel in the studied samples were acceptable and didn't exceed the standard value suggested by FAO/WHO and food and nutrition commission. About trace elements, cadmium was in the standard range. Lead value in the dried mint was exceeded the standard limit, significantly. As regards to, the only way of entrance of heavy metals to human body is not spices and other foods can contain them and growing spread of industry and technology, transportation progress, development of heavy metal mines, use of different chemical fertilizers for increasing the yield of agricultural products and running polluted swage to the agricultural lands and waters as well, the likelihood of increasing heavy metals in human food will increase. Thus, besides mentioned points, preventing pollution increase and training practitioners of agricultural products, the necessity of periodic examinations for estimating heavy metals especially lead in the most applicable herbal products such as spices and herbal medicines is emphasized.

Footnotes

Conflict of Interest:
The authors declared no conflict of interest.

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