Original Research

Determination of Cu, Mn, Hg, Pb, and Zn in the Outer Tissue Washings, Outer Tissues, and Inner Tissues of Different Vegetables Using ICP-OES

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Abstract

We have determined the levels of five heavy metals (Cu, Hg, Mn, Pb, Zn) in outer tissue washings, outer tissues, and inner tissues of different vegetable samples (beans, cabbage, capsicum, carrot, cauliflower, cucumber, eggplant, green pepper, pea, and tomato) from the Qassim region of Saudi Arabia. Instead of using the customary dried tissue power, we conducted all the analysis directly in the wet tissues following microwave digestion; the levels of heavy metals being reported in wet as well as dry tissue using the water-content factor. Pb was absent in the washings but present in most of the vegetables in the range of 0.013-0.251 μg/g wet tissue (0.201-5.055 μg/g dry tissue). Although traces of Hg (5.994-6.520 ng/g wet tissue) were present in the washing, this metal was not detected in the vegetable tissues. Cu was observed in the range of 0.079-1.785 μg/g wet tissue (1.104-22.919 μg/g dry tissue), whereas the range of Mn was found to be 0.239-3.263 μg/g wet tissue (4.626-47.036 μg/g dry tissue). Only in the outer tissues of peas were Cu levels found to be slightly higher (22.919 μg/g dried tissue) than the recommended upper limit of 20 μg/g. Zn was detected only in the beans, green pepper, and peas; its concentration was much higher in outer (552.77 μg/g) and inner (686.71 μg/g) tissues of green pepper than the recommended upper limit of 100 μg/g. The lower limits of detection (LOD) and quantification (LOQ) for Hg were found to be 0.81 and 1.61 ng/kg, respectively. The LOD and LOQ for the remaining heavy metals were as follows: Cu (1.28 and 4.27 μg/kg), Mn (0.23 and 0.77 μg/kg), Pb (7.56 and 24.79 μg/kg), and Zn (5.98 and 19.96 μg/kg). Our findings suggest that the analysis of heavy metals directly in the wet samples provides a quick alternative for screening of heavy metals as this method can determine the heavy metals well below their toxic limits. The presence of heavy metals in the washings of the outer tissues of vegetables points toward the importance of thoroughly washing vegetables before consumption.

Keywords: heavy metals, vegetables, toxicity, outer tissues, inner tissues, ICP

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Introduction

Several studies have reported unsafe and hazardous levels of heavy metals in vegetables for human consumption [1-4]. Prolonged consumption of unsafe concentrations of heavy metals in foodstuffs may lead to the disruption of numerous biological and metabolic processes in the human body. In the long run, accumulation of heavy metals in the body may reach toxic levels and pose such serious health effects as cancer, kidney damage, and developmental toxicity [5-7]. Epidemiological studies have revealed a high prevalence of systemic cancers in the regions where heavy metals are ubiquitous in environments polluted with industrial and agricultural wastes [8-11].

Heavy metals are one of the common types of contaminants that can be found on the surface and in the tissues of fresh vegetables. Contamination of vegetables with heavy metals may be due to irrigation with contaminated water, metal-based pesticides and fertilizers, industrial emissions, transportation, the harvesting process, and storage [12]. In addition, they could be contaminated when farmers wash them with polluted wastewater before bringing them to market. Sharma et al. [2] have suggested that transportation and marketing systems of vegetables play a significant role in elevating the contaminant levels of heavy metals, which may pose a threat to the quality of the vegetables. Mor and Ceylan [4] have observed higher levels of Pb and Cd in the vegetables grown in traffic areas than those found in rural areas. Vegetables grown at contaminated sites could take up and accumulate metals at concentrations that are toxic [13].

As plants constitute the foundation of the food chain, some concerns have been raised about the possibility of toxic concentrations of certain elements being transported from plants to higher strata of the food chain [14]. It is notable that vegetables, consumed raw or cooked, pose similar hazards because the cooking process is ineffective in reducing metal concentrations [15]. Heavy metals may be present as a deposit of the surface of the vegetable, or may be taken up by the crop roots and incorporated into the plant tissue. This distinction is very important, because metal deposited on the surface of the crop can often be washed off by consumers prior to their consumption.

In view of these facts, we aimed to investigate the heavy metal levels in the outer tissue washings, outer tissues and inner tissues of commonly used vegetables. We have conducted all the analysis on wet samples rather than using the customary dried tissues to investigate the practicality of this strategy for routine application. The significance of of this study is directly related to the safeguard of human beings from the health hazards associated with the consumption of heavy metals-contaminated vegetables. The analytical methodology reported in this study can successfully determine the levels of heavy metals in the vegetables, several-fold below their toxic limits.

Materials and Methods

Sample Collection

The vegetable samples (beans, cabbage, capsicum, carrot, cauliflower, cucumber, eggplant, green pepper, pea, and tomato) were collected in January from the wholesale market of Qassim city at the entry point to avoid further modifications or multiple cleanings by retailers. These vegetables arrived from different farming locations in the vicinity of Qassim. The samples were individually placed in polythene bags and transported to a lab, where they were kept frozen until analyzed.

Sample Preparation

The vegetable samples were subjected to three different types of sample preparation modes:

(a) outer tissue, including the outer surface
(b) inner tissue, including the deep core region
(c) washing of the outer tissue

For categories ‘a’ and ‘b’, 0.5 g of wet tissue was acid digested (5 ml HNO_3 + 1 ml HCl) in a microwave. For category 'c', 0.5 g of outer tissue was transferred to a vial containing 2 ml of 5% HNO_3. The vial was shaken in an orbital shaker for 5 min, centrifuged at 14,000 rpm for 5 min and the 1.5 ml of supernatant was stored for analysis. All the samples were analyzed in triplicate.

Microwave Digestion

The vegetable samples (0.5 g of wet tissue) were individually placed in pre-cleaned Teflon tubes of the MF-100 rotor (Anton Parr, Austria), followed by the addition of 6 ml of acid mixture (5 ml HNO_3 + 1 ml HCl). The tubes were securely placed in the jacket, capped and evenly arranged in the rotor stand while one tube contained the pressure-temperature sensor. Samples underwent pressurized digestion in an automated microwave digestion system (Anton Parr). The digestion temperature and pressure were set to 160ºC and 20 bar, respectively. The digestion program was conducted using the power of 1,400 W, ramp time of 10 min., hold time of 15 min., and cooling time of 15 min.

Elemental Analysis

The elemental analysis was performed on ICP Model DV7000 (Perkin Elmer, USA). Prior to analysis, all the samples were diluted 10-fold with ultra pure de-ionized water. For Hg analysis, a FIAS system (Perkin Elmer, USA) was used and the standards and samples were stabilized by adding 1 drop of 5% (w/v) $KMnO_4$ solution. For FIAS, 0.02% $NaBH_4$ in 0.05% NaOH was used as reductant and 3% HCl as a carrier. The limits of detection (LOD) and quantification (LOQ) were calculated as the blank signal plus three or ten times its standard deviation, respectively [16].

Table 1. Initial wet weight and final dry weight of vegetables samples (average of 3 samples).

Vegetable	Outer tissue				Inner tissue			
	IW (g)	FW (g)	IW/FW	Water (%)	IW (g)	FW (g)	IW/FW	Water (%)
Beans	10	1.036	9.652	89.64	10	0.927	10.787	90.73
Cabbage	10	0.820	12.195	91.80	10	0.440	22.727	95.60
Capsicum	10	1.162	8.605	88.38	10	0.962	10.395	90.38
Carrot	10	0.646	15.479	93.54	10	0.992	10.080	90.08
Cauliflower	10	0.688	14.534	93.12	10	0.712	14.044	92.88
Cucumber	10	0.380	26.315	96.20	10	0.359	27.855	96.41
Eggplant	10	0.855	11.695	91.45	10	0.397	25.188	96.03
Pepper	10	0.536	18.656	94.64	10	0.271	36.900	97.29
Pea	10	0.876	14.415	91.24	10	1.392	7.183	86.08
Tomato	10	0.798	12.531	92.02	10	0.426	23.474	95.74

IW – initial wet weight, FW – final dry weight

Table 2. Heavy metals concentration (expressed as ng/g wet tissue for Hg and µg/g wet tissue for other elements ± standard deviation) in the washings* of outer tissues of the vegetables.

Vegetable	Cu	Hg	Mn	Pb	Zn
Beans	0.441±0.053	6.288±0.012	1.421±0.094	ND	1.582±1.235
Cabbage	0.047±0.045	6.274±0.003	0.633±0.189	ND	1.317±1.099
Capsicum	0.430±0.201	6.414±0.117	1.607±0.792	ND	0.806±0.301
Carrot	0.352±0.120	6.066±0.047	2.019±0.785	ND	1.623±0.811
Cauliflower	0.008±0.005	6.191±0.055	1.654±0.357	ND	1.173±0.530
Cucumber	0.525±0.163	5.994±0.032	1.151±0.494	ND	1.403±1.309
Eggplant	0.208±0.073	6.520±0.211	1.554±0.510	ND	3.267±0.751
Green pepper	0.209±0.050	6.276±0.011	0.905±0.442	ND	1.109±0.104
Pea	0.693±0.318	6.289±0.078	1.579±1.251	ND	7.668±2.823
Tomato	3.297±1.013	6.328±0.002	0.688±0.093	ND	1.610±0.081

*Please refer to methods section for details of washing protocol. ND – not detected.

Statistics

The data of heavy metals in the washings were analyzed by one-way analysis of variance (ANOVA). The levels of heavy metals in the outer and inner tissues were compared using Student's t-test. The *P* values <0.05 were considered as statistically significant.

Results

The initial wet weight and the final dry weight of vegetable samples following 48 h drying at 65ºC is given in Table 1. The water content was high in the inner tissues of vegetables except for carrot, cauliflower, and pea, which had slightly higher levels of water in the outer tissues (Table 1).

In the washing of outer tissues of vegetables, the range of various elements was as follows: Cu (0.008-3.297 µg/g, ANOVA F=22.39, P<0.001), Hg (5.994-6.520 ng/g, ANOVA F=9.71, P<0.001), Mn (0.633-2.019 µg/g, ANOVA F=1.71, P=0.154), and Zn (0.806-7.668 µg/g, ANOVA F=9.54, P<0.001). Pb was not detected in the washings (Table 2).

The concentration of heavy metals in the outer and inner wet tissues is given in Table 3. The same concentration data (µg/g wet tissue) was converted to conventional units (µg/g dry tissue) (Table 4) using IW/FW conversion factor (Table 1) for respective vegetable samples. Hg was not detected in any of the vegetable samples irrespective of tissue type – outer or inner.

The acceptable upper limits of heavy metals have been reported as Cu (20 µg/g), Mn (20 µg/g), Pb (9 µg/g), and

Table 3. Heavy metals concentrations (µg/g wet tissue ± standard deviation) in outer and inner tissues of vegetable samples.

Vegetable	Tissue	Cu	Mn	Pb	Zn
Beans	Outer	0.686±0.125	2.811±0.501	0.127±0.221	5.940±6.363
	Inner	0.644±0.092	0.908±0.557*	0.111±0.068	2.281±1.730
Cabbage	Outer	ND	1.318±0.514	ND	ND
	Inner	0.091±0.097	0.666±0.472	0.251±0.059**	ND
Capsicum	Outer	0.256±0.009	2.241±0.165	0.079±0.137	ND
	Inner	1.239±1.697	0.551±0.189***	0.207±0.143	ND
Carrot	Outer	0.079±0.096	2.418±0.731	0.013±0.022	ND
	Inner	ND	0.459 ±0.045**	0.050±0.087	ND
Cauliflower	Outer	0.076±0.118	3.000±0.010	ND	ND
	Inner	0.399±0.424	1.506±0.093***	0.052±0.001	ND
Cucumber	Outer	0.517±0.151	1.342±0.032	0.186±0.112	ND
	Inner	ND**	0.239±0.142***	0.083±0.143	ND
Eggplant	Outer	ND	1.430±0.614	ND	ND
	Inner	ND	0.699±0.207	ND	ND
Green pepper	Outer	0.154±0.162	1.504±0.631	0.093±0.057	29.63±2.595
	Inner	ND	ND*	0.137±0.124	18.61±2.55**
Pea	Outer	1.590±0.315	3.263±2.020	0.074±0.128	6.067±2.352
	Inner	1.785±0.736	1.449±0.552	0.150±0.165	9.480±4.655
Tomato	Outer	0.716±0.302	0.837±0.200	ND	ND
	Inner	ND*	ND**	0.057±0.069	ND

*P<0.05, **P<0.01, and ***P<0.001 versus outer tissue using Student t-test. ND – not detected.

Zn (100 µg/g [17]; Cu (10 µg/g), Pb (1.5 µg/g) and Zn (150 µg/g) [18]; Prevention of Food adulteration (PFA) act standards for Cu (30 µg/g), Pb (2.5 µg/g) and Zn (50 µg/g [19]; and the European Union (EU) standard for Pb (0.3 µg/g) [20].

The level of Cu was comparatively higher in the inner tissues of beans, cabbage, capsicum, and cauliflower than their outer tissue counterparts, but the reverse was true for carrot, cucumber, green pepper, peas, and tomato (Tables 3 and 4). Only in the outer tissues of peas were Cu levels found to be slightly higher (22.919 µg/g dried tissue) than the recommended upper limit of 20 µg/g [17]. In all the vegetables, Mn levels were higher in the outer tissues than the respective inner tissues; these differences were statistically significant in 7 vegetables (Tables 3 and 4). The concentration of Mn exceeded the recommended upper limit of 20 µg/g in the outer tissues of beans (27.131 µg/g), carrot (37.402 µg/g), cauliflower (43.602 µg/g), cucumber (35.314 µg/g), green pepper (28.058 µg/g), and pea (47.036 µg/g). However, only the inner tissues of cauliflower had marginally higher Mn levels (21.150 µg/g) than the recommended limit (Table 4). Pb was not detected in the outer tissues of cabbage, cauliflower, and tomato, but was present in the inner tissues of these vegetables. In all the vegetables, the levels of Pb were much below the recommended upper limit (Table 4). Zn was detected only in beans, green pepper, and peas; the outer tissues of beans and peas had higher levels of Zn than the inner tissues, whereas the reverse was true for green pepper (Table 4). The concentration of Zn was much higher in outer (552.77 µg/g) and inner (686.71 µg/g) tissues of green pepper than the recommended upper limits of 50-150 µg/g [17-20].

The LOD and LOQ for Hg were found to be 0.81 ng/kg and 1.61 ng/kg, respectively. The LOD and LOQ for the remaining heavy metals were, respectively, as follows: Cu (1.28 µg/kg and 4.27 µg/kg), Mn (0.23 µg/kg and 0.77 µg/kg), Pb (7.56 µg/kg and 24.79 µg/kg), and Zn (5.98 µg/kg and 19.96 µg/kg).

Discussion

Vegetables are the essential components of our healthy diet. Humans are encouraged to consume more vegetables because they serve as a cheap and efficient source of vitamins, minerals and fiber. But it is important to note that vegetables contain both essential and toxic metals over a wide range of concentrations, depending on the surround-

Table 4. Heavy metals concentrations (μg/g dry tissue±standard deviation; computed using Tables 2 and 3 data) in outer and inner tissues of vegetable samples.

Vegetable	Tissue	Cu	Mn	Pb	Zn
Beans	Outer	6.621±1.206	27.131±4.835	1.225±2.133	57.332±61.41
	Inner	6.946±0.992	9.794±6.008*	1.197±0.733	24.605±18.66
Cabbage	Outer	ND	16.073±6.268	ND	ND
	Inner	2.068±2.204	15.136±10.727	5.704±1.340**	ND
Capsicum	Outer	2.202±0.077	19.283±1.419	0.679±1.178	ND
	Inner	12.879±17.64	5.727±1.964**	2.151±1.486	ND
Carrot	Outer	1.222±1.485	37.402±11.315	0.201±0.340	ND
	Inner	ND	4.626±0.453**	0.504±0.876	ND
Cauliflower	Outer	1.104±1.715	43.602±0.145	ND	ND
	Inner	5.603±5.954	21.15±1.306***	0.730±0.014***	ND
Cucumber	Outer	13.604±3.973	35.314±0.842	4.894±2.947	ND
	Inner	ND**	6.657±3.955***	2.311±3.983	ND
Eggplant	Outer	ND	16.723±7.180	ND	ND
	Inner	ND	17.606±5.213	ND	ND
Green pepper	Outer	2.873±3.022	28.058±11.771	1.735±1.063	552.77±48.41
	Inner	ND	ND*	5.055±4.575	686.71±94.09
Pea	Outer	22.919±4.540	47.036±29.118	1.066±1.845	87.455±39.90
	Inner	12.821±5.286	10.408±3.965	1.077±1.185	68.094±33.43
Tomato	Outer	8.972±0.378	10.488±2.506	ND	ND
	Inner	ND***	ND**	1.338±1.619	ND

*P<0.05, **P<0.01, and ***P<0.001 versus outer tissue using Student t-test. ND – not detected. The acceptable upper limits (μg/g) of heavy metals in vegetables have been reported as Cu, 10-30; Mn, 20; Pb, 0.3-9; Zn, 50-150 [17-20].

ing environmental conditions [2]. Plants largely take up metals by directly absorbing them from the contaminated soil as well as from the deposits on parts of the plants exposed to polluted air [21]. Contaminated sediments are one of the several means through which soils are enriched with heavy metals [22]. Use of wastewater and sludge application in agricultural lands enriches soils with heavy metals to concentrations that may pose potential environmental and health risks by contaminating the vegetable crops in the long run [23]. Several investigators have shown that some common vegetables are capable of accumulating high levels of metals from the soil [24-26]. Prolonged consumption of vegetables with elevated heavy metals concentration causes several health risks, while pregnant women or very young children are highly vulnerable to heavy metal toxicity [27]. Owing to cumulative persistency and potential toxicity of heavy metals, it is important to analyze them in commonly used vegetables to ensure the levels of these contaminants meet agreed international requirements [28].

Among the five heavy metals analyzed in this study, Cu, Mn, and Zn act as micronutrients for the growth of humans and animals when present in trace quantities, whereas Hg and Pb are potentially toxic and cause serious human health hazards. In this investigation, we used microwave digestion, which is a rapid and efficient method for sample decomposition prior to the determination of heavy metals [29]. Soylak et al. [30] compared the dry ashing, wet ashing, and microwave digestion methods for sample preparation and found the microwave method as the best for sample digestion before heavy metals analysis, with recoveries of ≥98%. For the determination of heavy metals in microwave-assisted acid-digested samples, we used the technique of ICP-OES, which offers a rapid and convenient protocol for multi-element analysis as compared to conventional atomic absorption spectrometry.

The results of our study showed that although Hg was present in the washings, it was not detected in vegetable tissues. On the other hand, Pb was absent in the washings but distributed in variable amounts in the outer and inner tissues of different vegetables. Zn was present in the washings from all the ten vegetables, but only three vegetables showed the presence of Zn in their tissues. Other elements also showed variable patterns in the outer and inner tissues of different vegetables (Tables 3 and 4).

In plants, accumulation or enrichment factor (EF) as well as transfer factor (TF) varies depending upon the species and plant part [31]. The low levels of Pb in vegetables may be due to low TF values for Pb and, therefore, its minimal bioavailability [31]. Moreover, soil amendments such as zeolite, compost, and mesoporous molecular sieves have been shown to modify the uptake of heavy metals (Cd, Cu, Pb) by the plant shoots [32]. We observed very low levels of Cu in some vegetables including cabbage, carrot, and eggplant (Table 4). Itanna [33] have also reported deficient ranges in cabbage in vegetables at two agricultural sites. One possible deficiency of Cu in plant tops could be due to its preferential accumulation in roots [34]. Although the levels of Mn in outer tissues of vegetables including beans, carrot, cauliflower, cucumber, green pepper, and peas was higher than the recommended upper limit, it may not pose serious concern because the concentration on Mn in the inner tissues (that makes the bulk) was comparatively much less (Table 4). Similarly, the exceptionally high level of Zn in green pepper may not be considered hazardous as this vegetable is consumed in a very limited amount. Zinc is the least toxic element that is essential for human diet. It is required to maintain the proper functions of the immune system and is particularly important for normal brain activity as well as fetal growth and development. Thus, Zn deficiency in the diet could be more detrimental to human health than occasional consumption of moderately high levels of this metal.

In conclusion, the vegetables available in the local market of the Qassim region are safe to eat because the levels of common heavy metals therein are below the recommended upper limits. In particular, they are free from Hg and contain only traces of Pb. The detection of heavy metals in the washings of the outer tissues of vegetables point toward the importance of thorough washing of vegetables before consumption. All the analysis was conducted in wet samples that fairly assayed the heavy metals well below their toxic limits, hence offering a quick strategy for screening of heavy metals in vegetables.

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